

Cotransfection of 293Cre cells with pBHG10lox and  
a "Lox" shuttle plasmid for generation of Ad expression vectors

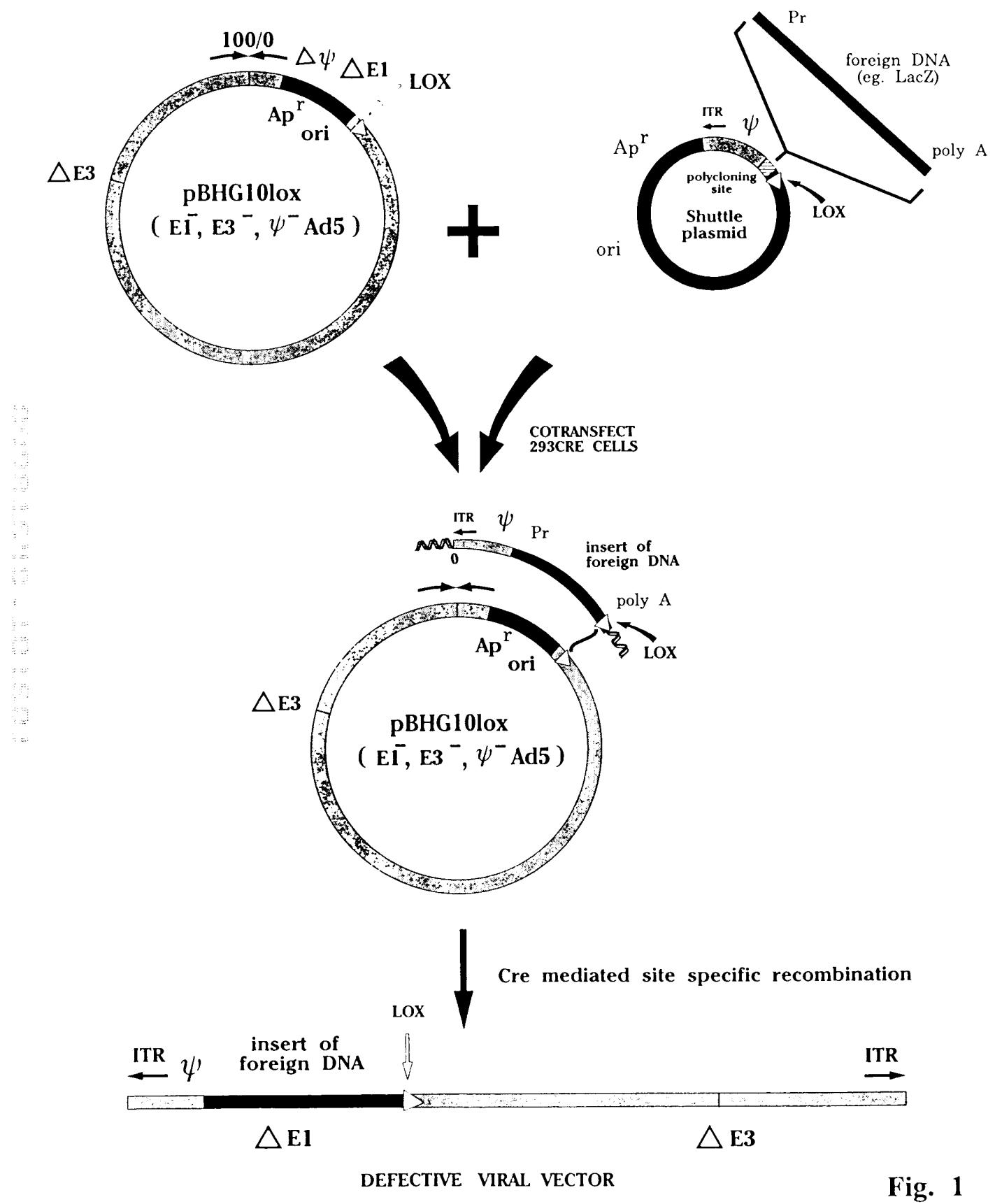


Fig. 1

**Cotransfection of 293Cre cells with pBHG10lox and a "lox" shuttle plasmid for generation of Ad expression vectors**

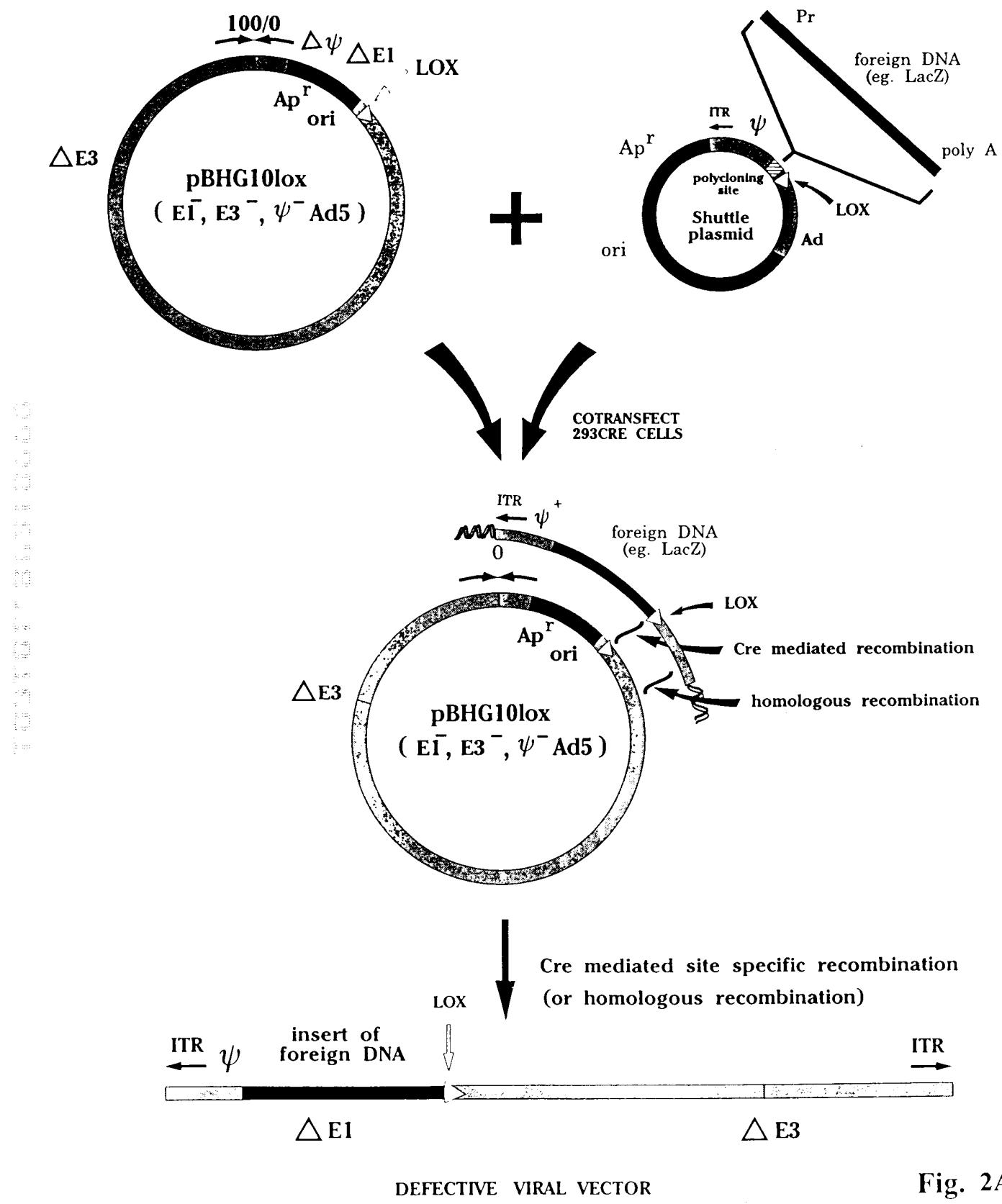


Fig. 2A

## CONSTRUCTION OF VARIOUS SHUTTLE PLASMIDS

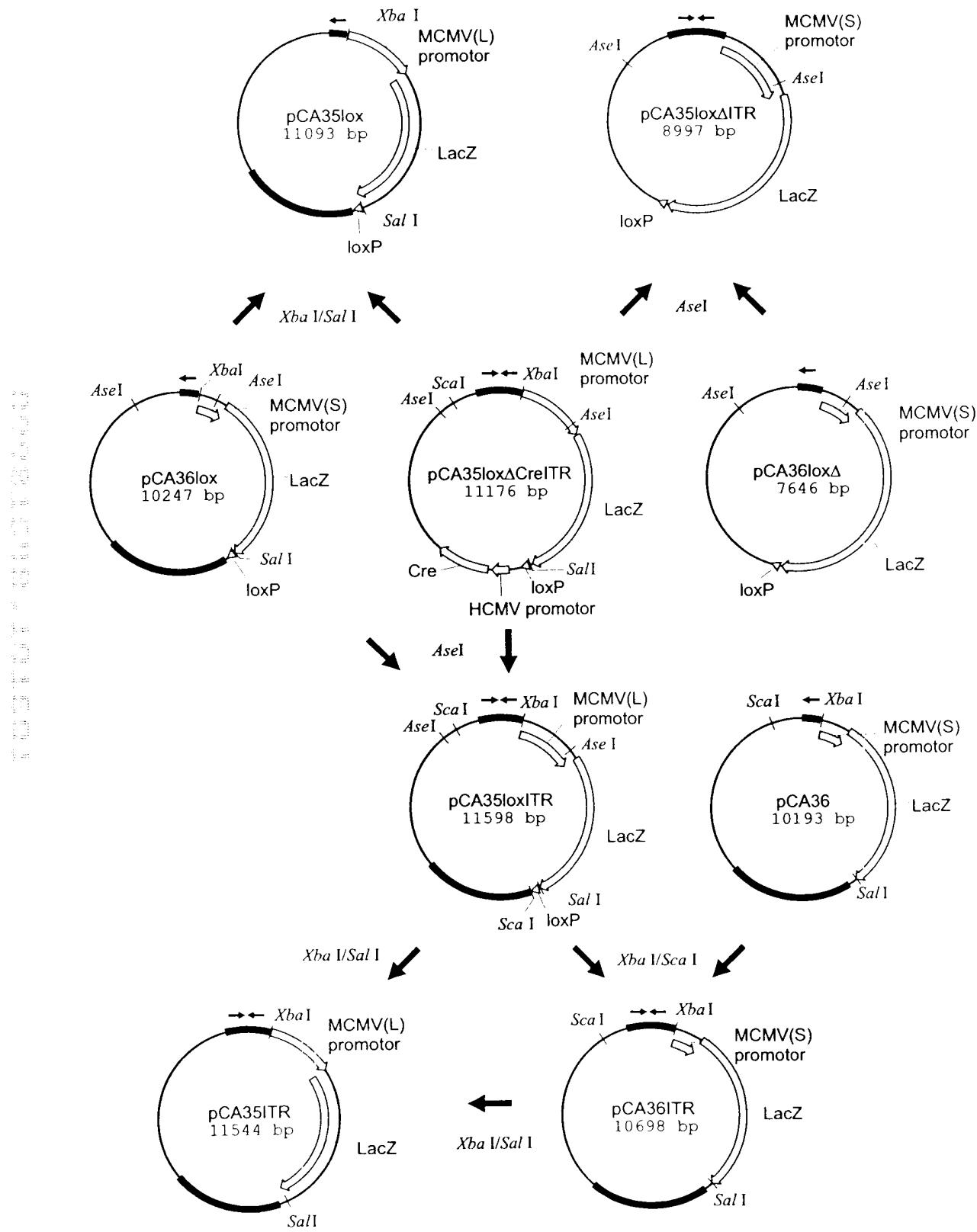


Figure 2B

# OLIGONUCLEOTIDES USED IN CLONING

## AB3233/3234 : loxP linker

SEQ. ID. NO. : 1

loxP site

*Bam*H I/*Bgl* II  
overhang

5' GATCCAATAACTCGTATAGCATACATTATACGAAGTTATAAGTACTGAATTG 3'  
3' GTTATTGAAGCATATCGTATGTAATATGCTCAATATTGACTTAAGCCTAG 5'

*Bam*H I/*Bgl* II  
overhang

SEQ. ID. NO. : 2

## AB14626/14627 : Multiple Cloning Site

SEQ. ID. NO. : 3

*Sma* I      *Bgl* II      *Hind* III      *Sac* I

*Sal* I overhang

5' AATTCCCCGGGAGATCTAAGCTTGAGCTCG 3'  
3' GGGGCCCTCTAGATTGAACTCGAGCAGCT 5'

*Eco*R I overhang

SEQ. ID. NO. : 4

## AB6920/6921 : loxP linker

SEQ. ID. NO. : 5

*Xba* I overhang

5' CTAGCAATAACTCGTATAGCATACATTATACGAAGTTATATCGATG 3'  
3' GTTATTGAAGCATATCGTATGTAATATGCTCAATATAGCTACGATC 5'

*Xba* I overhang

SEQ. ID. NO. : 6

## AB14680/14681 : loxP linker

SEQ. ID. NO. : 7

*Bln* I overhang

*Cla* I

5' TGACAATAACTCGTATAGCATACATTATACGAAGTTATATCGATG 3'

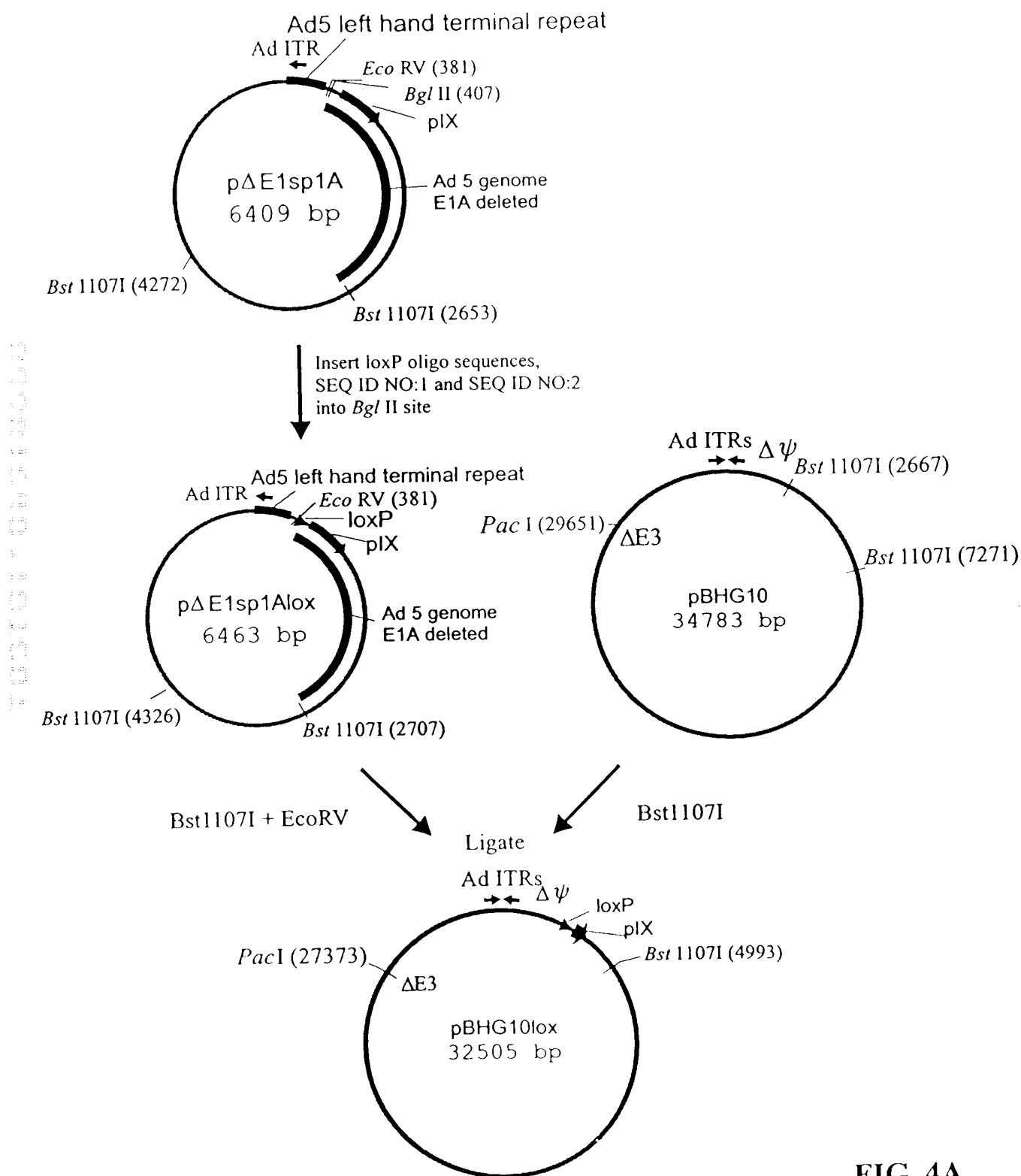
3' GTTATTGAAGCATATCGTATGTAATATGCTCAATATAGCTACACT 5'

*Bln* I overhang

SEQ. ID. NO. : 8

Fig. 3

# CONSTRUCTION OF A CIRCULAR GENOMIC PLASMID FOR Ad VECTOR RESCUE USING THE Cre/ loxP SYSTEM



**FIG. 4A**

## CONSTRUCTION OF pBHGdX1P<sub>lox</sub>

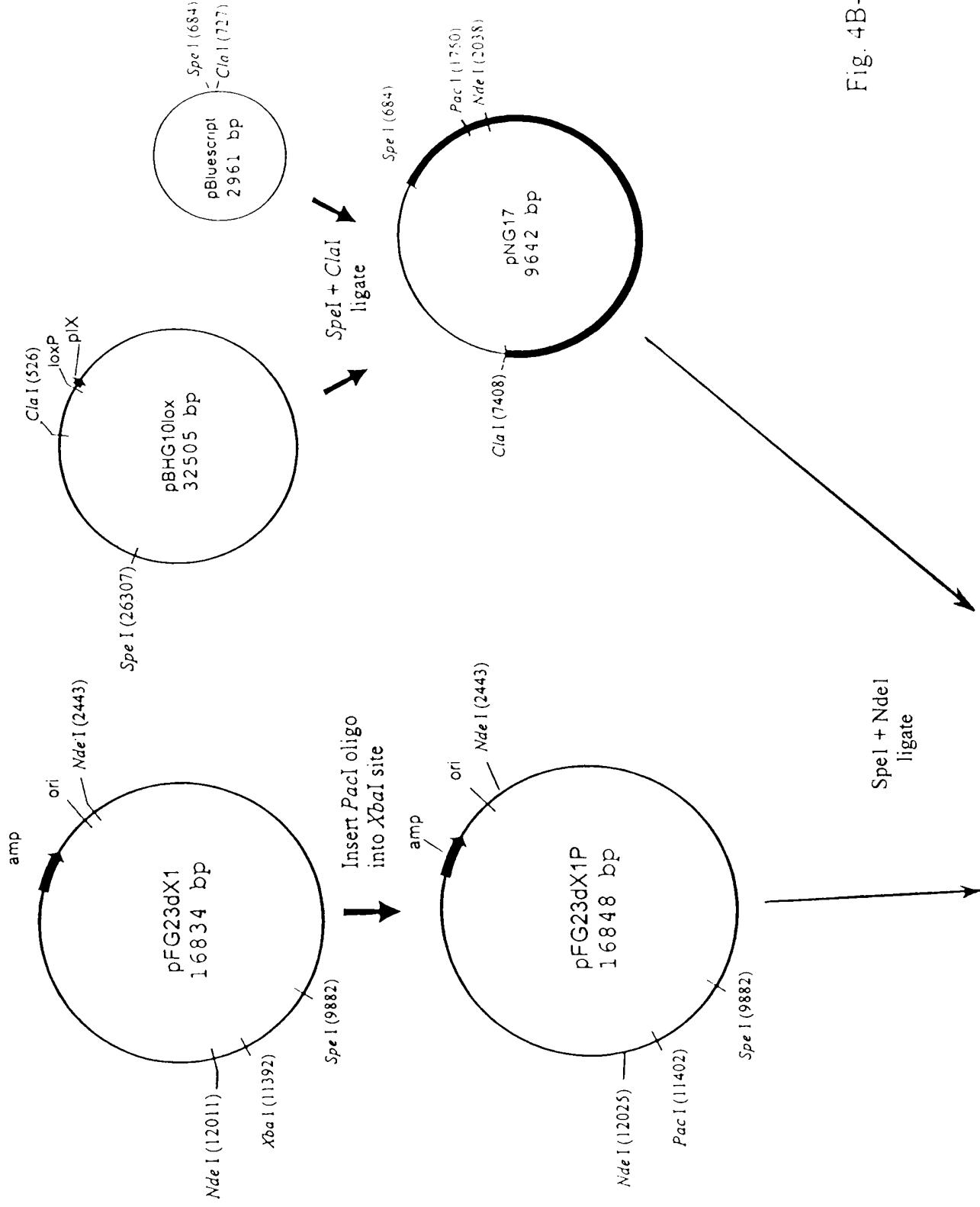


Fig. 4B-1

CONSTRUCTION OF pBHGdX1P<sub>lox</sub>

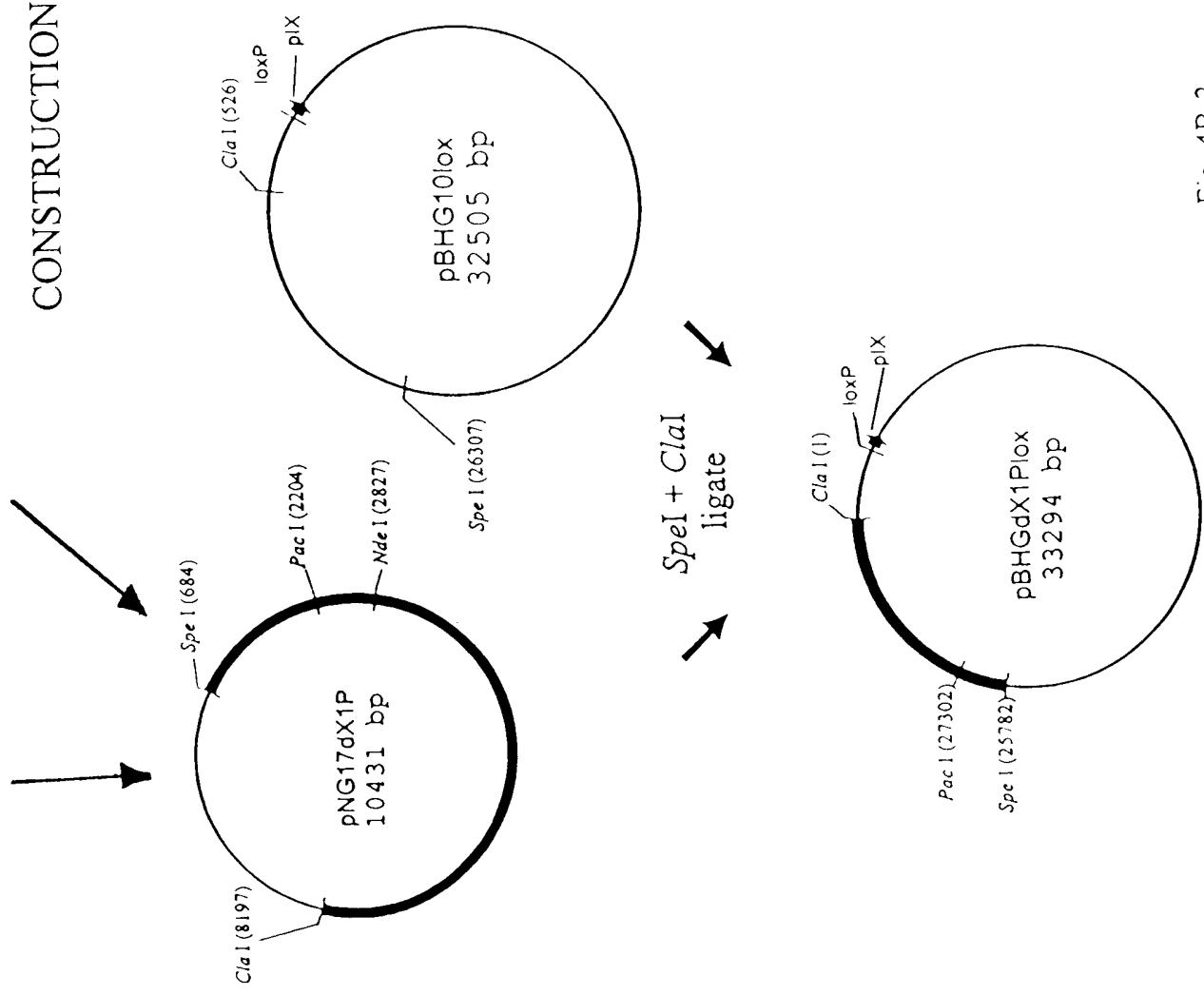


Fig. 4B-2

# CONSTRUCTION OF pBHGE3lox

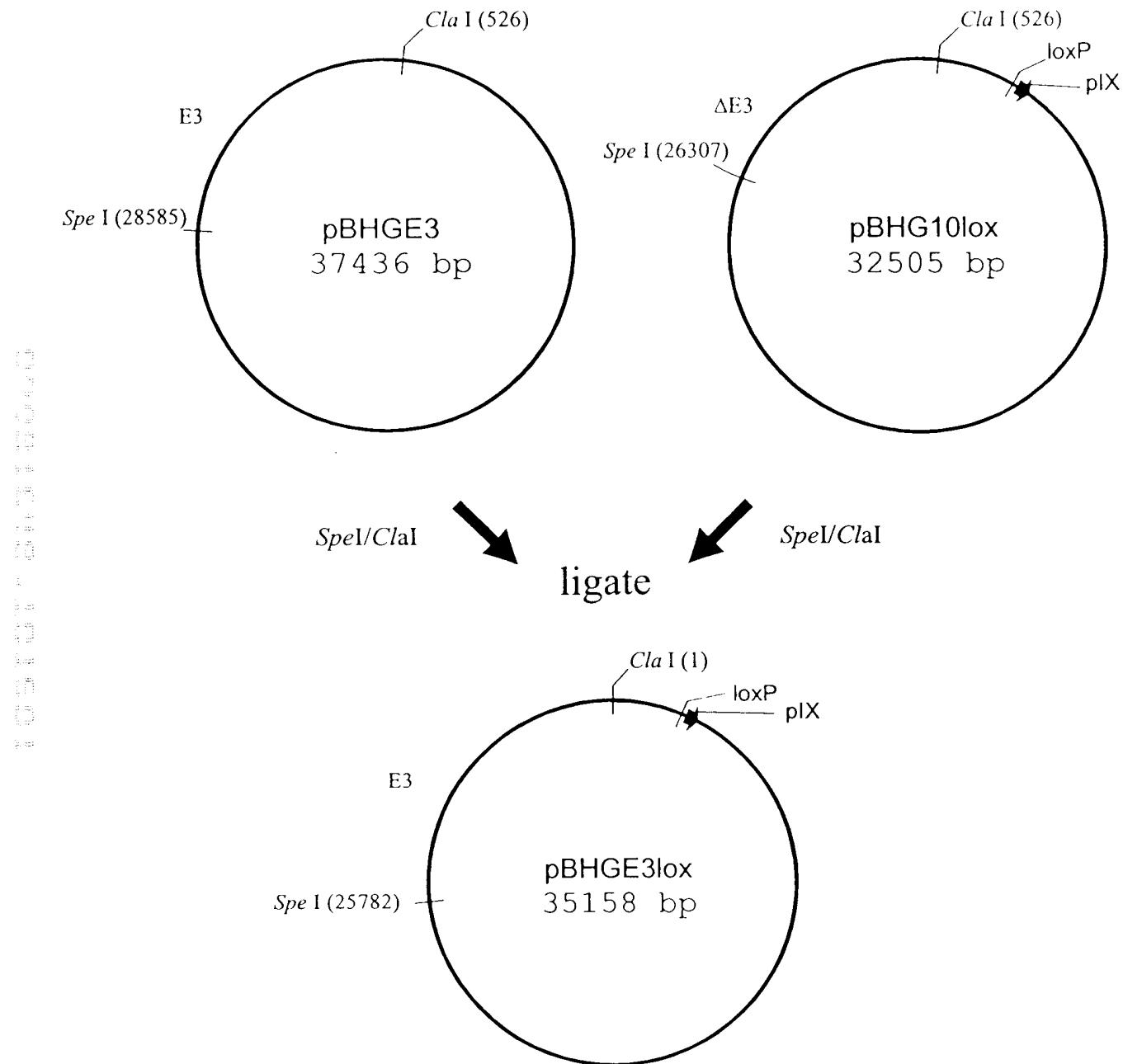


Fig. 4C

## CONSTRUCTION OF Ad GENOMIC PLASMIDS ENCODING CRE

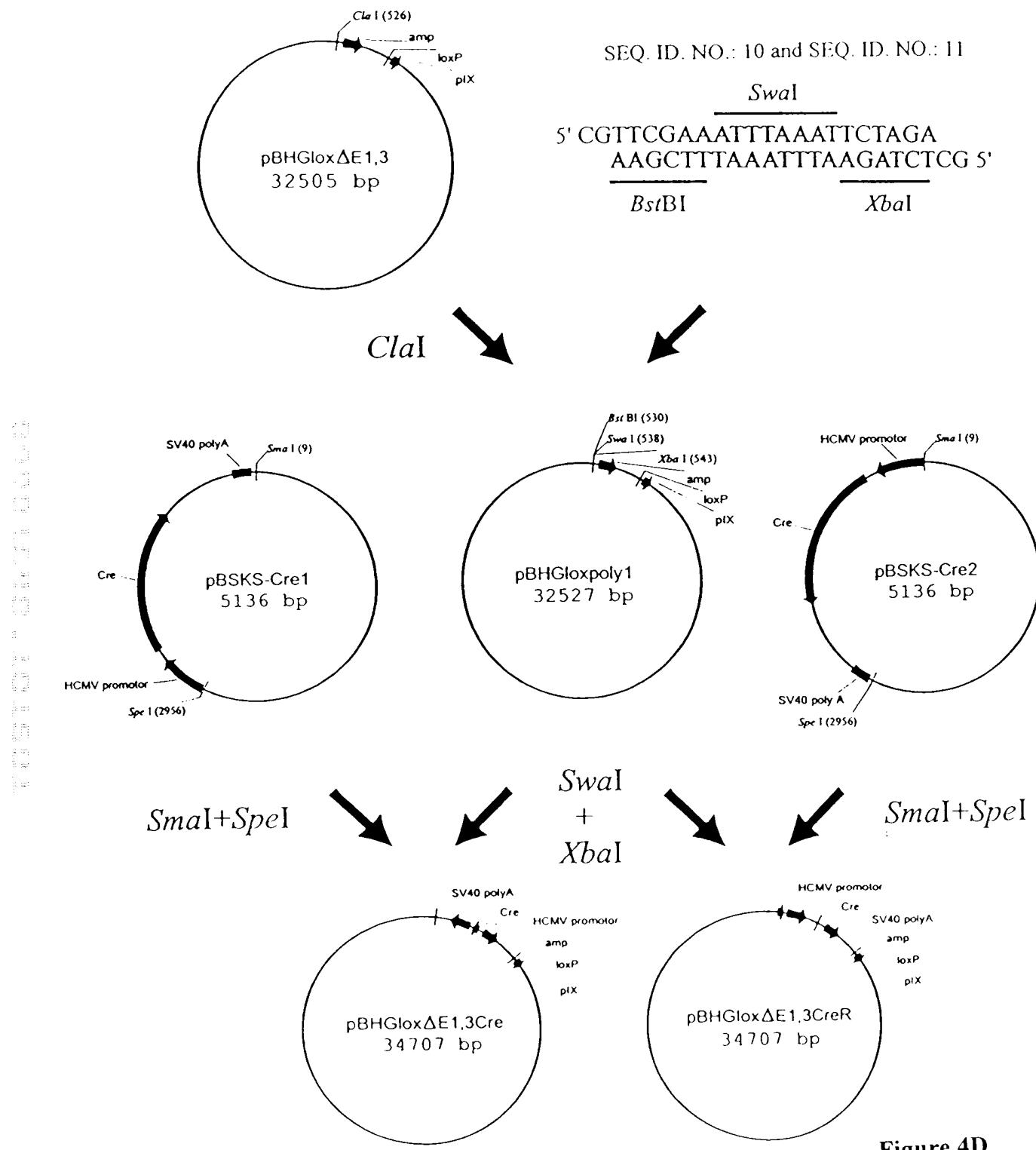


Figure 4D

# CONSTRUCTION OF pΔE1SP1A & pΔE1SP1B loxP PLASMIDS FOR RESCUE OF FOREIGN DNA

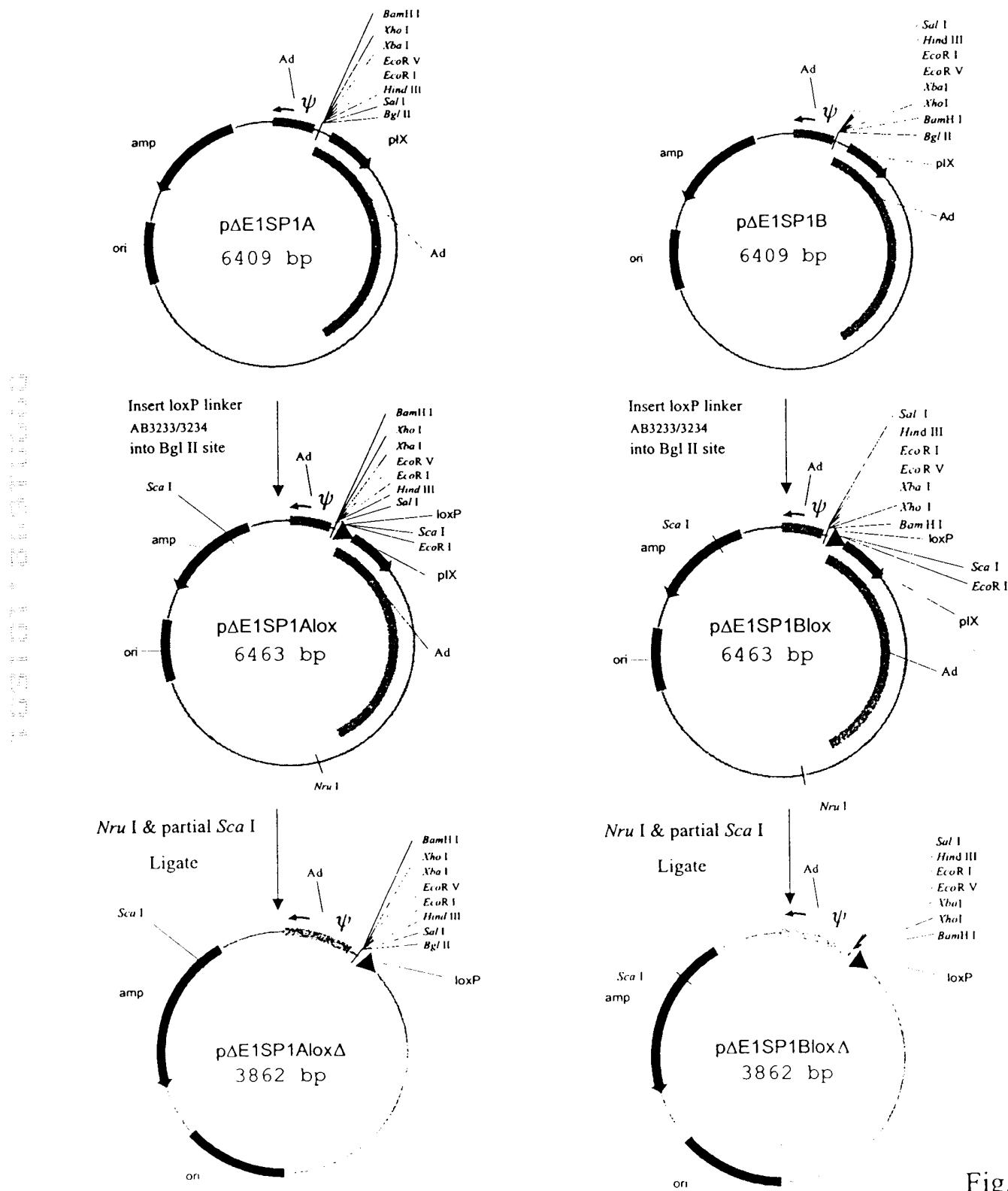


Fig. 5A

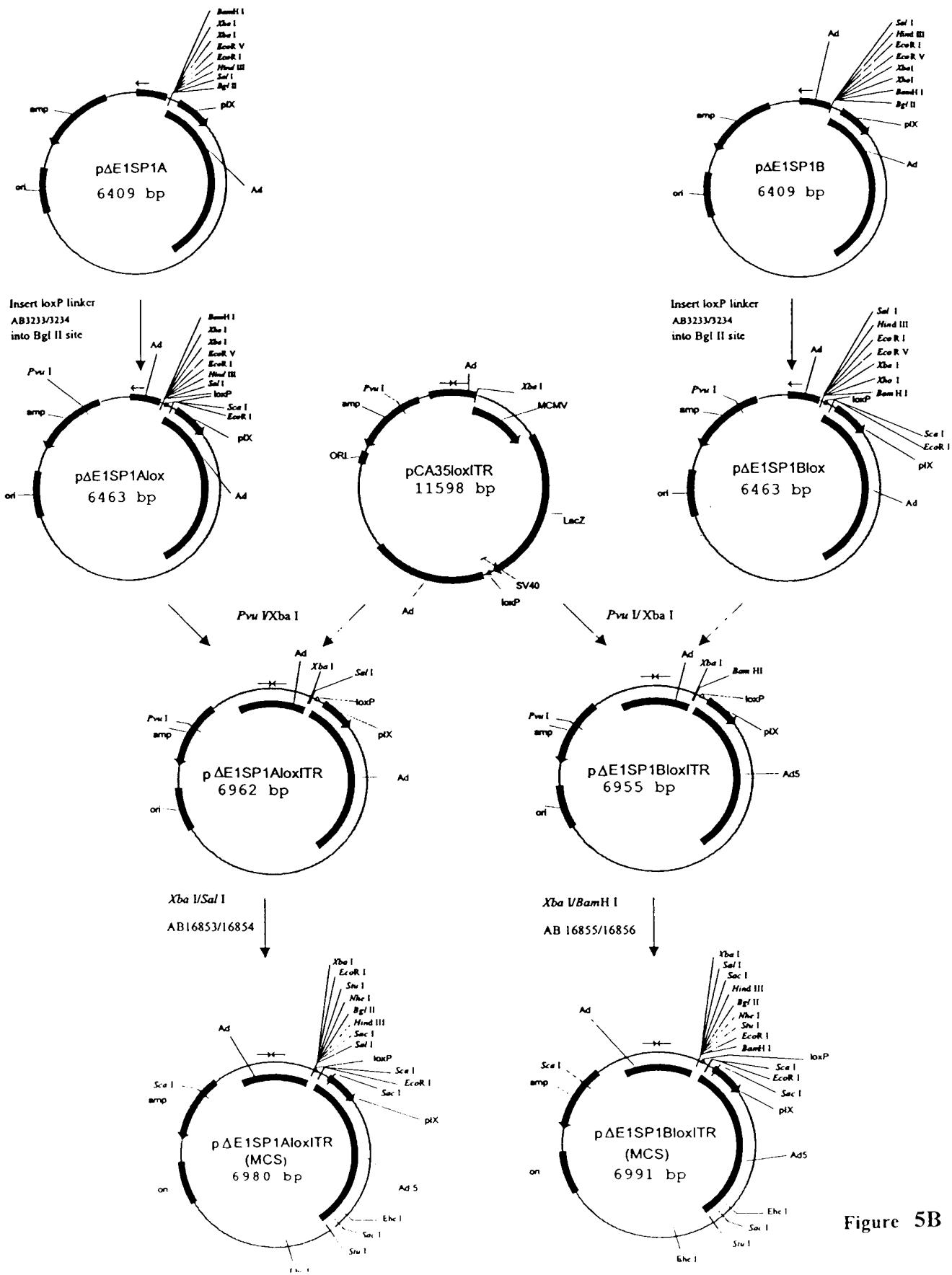
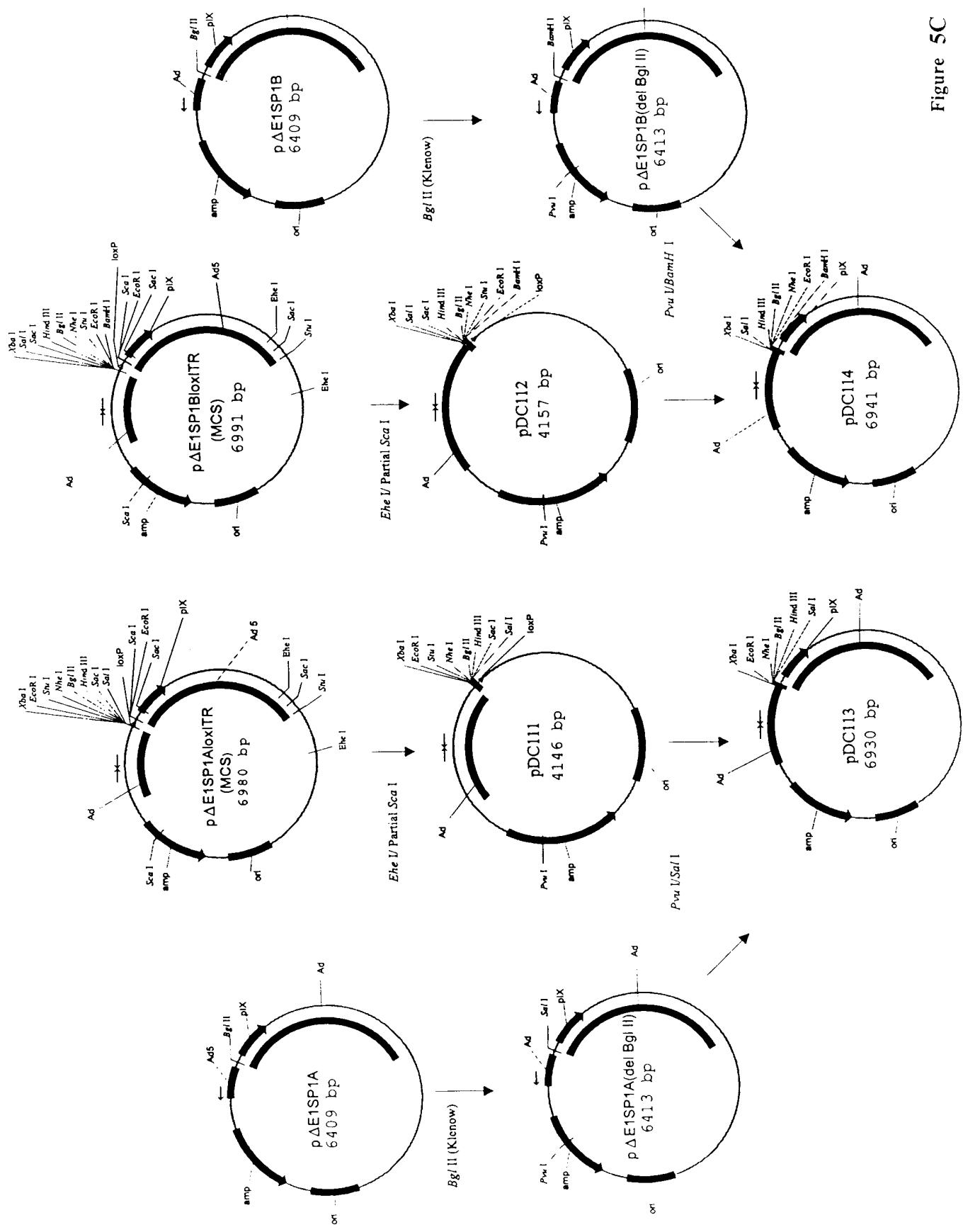
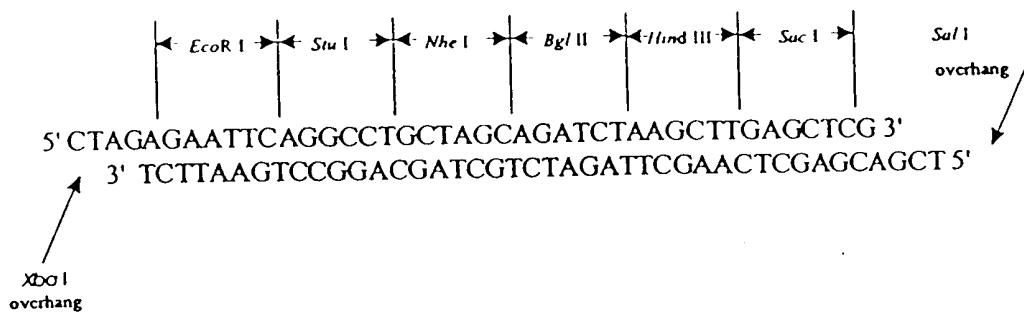


Figure 5B

**Figure 5C**



SEQ. ID. NO.: 12 (AB16853) and SEQ. ID. NO.: 13 (AB16854)



SEQ. ID. NO.: 14 (AB16855) and SEQ. ID. NO.: 15 (AB16856)



Figure 5D

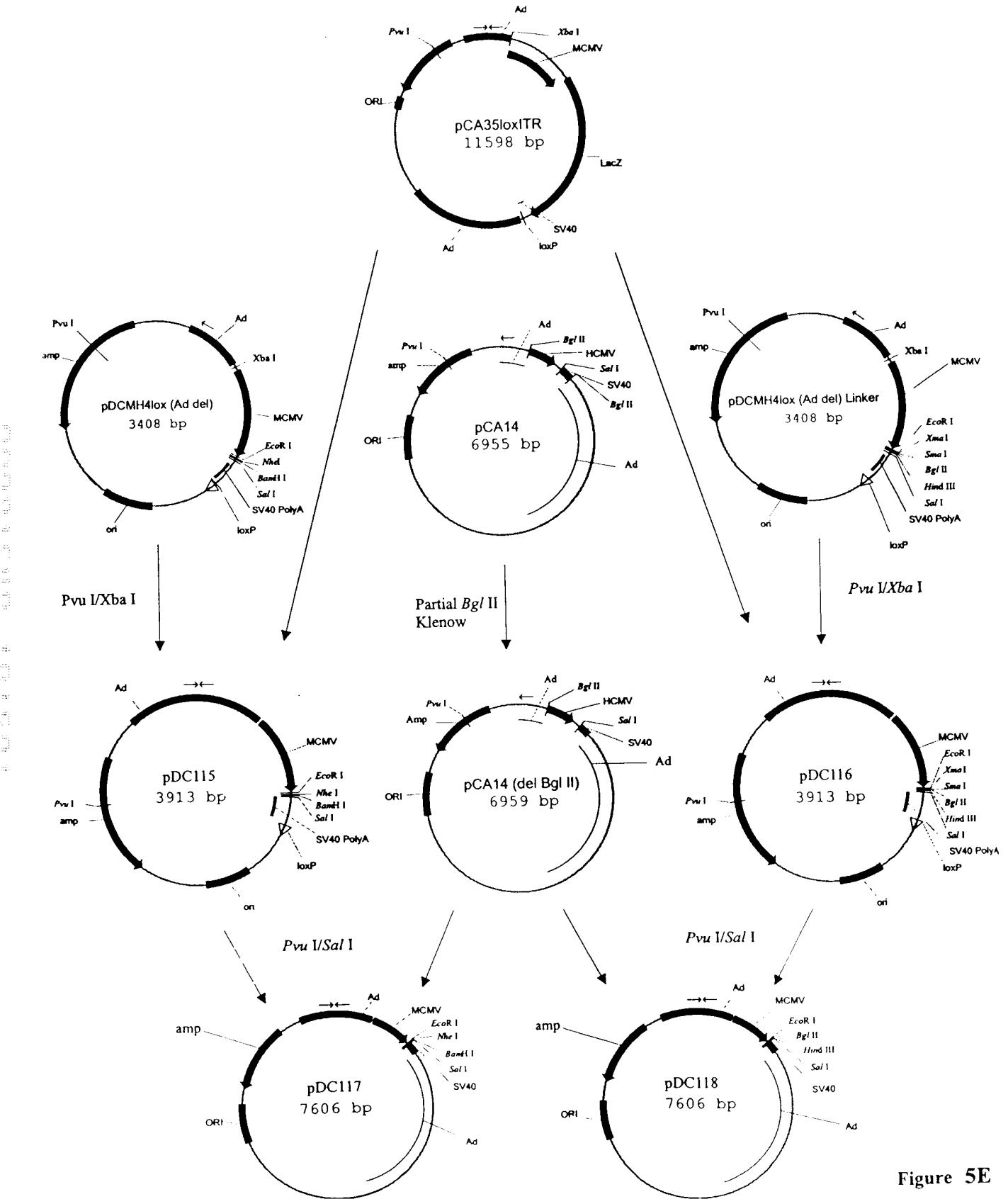


Figure 5E

# CONSTRUCTION OF pMH4LOX, pMH4LOX $\Delta$ and pMH4LOX $\Delta$ LINK SHUTTLE PLASMIDS FOR RESCUE OF EXPRESSION CASSETTES

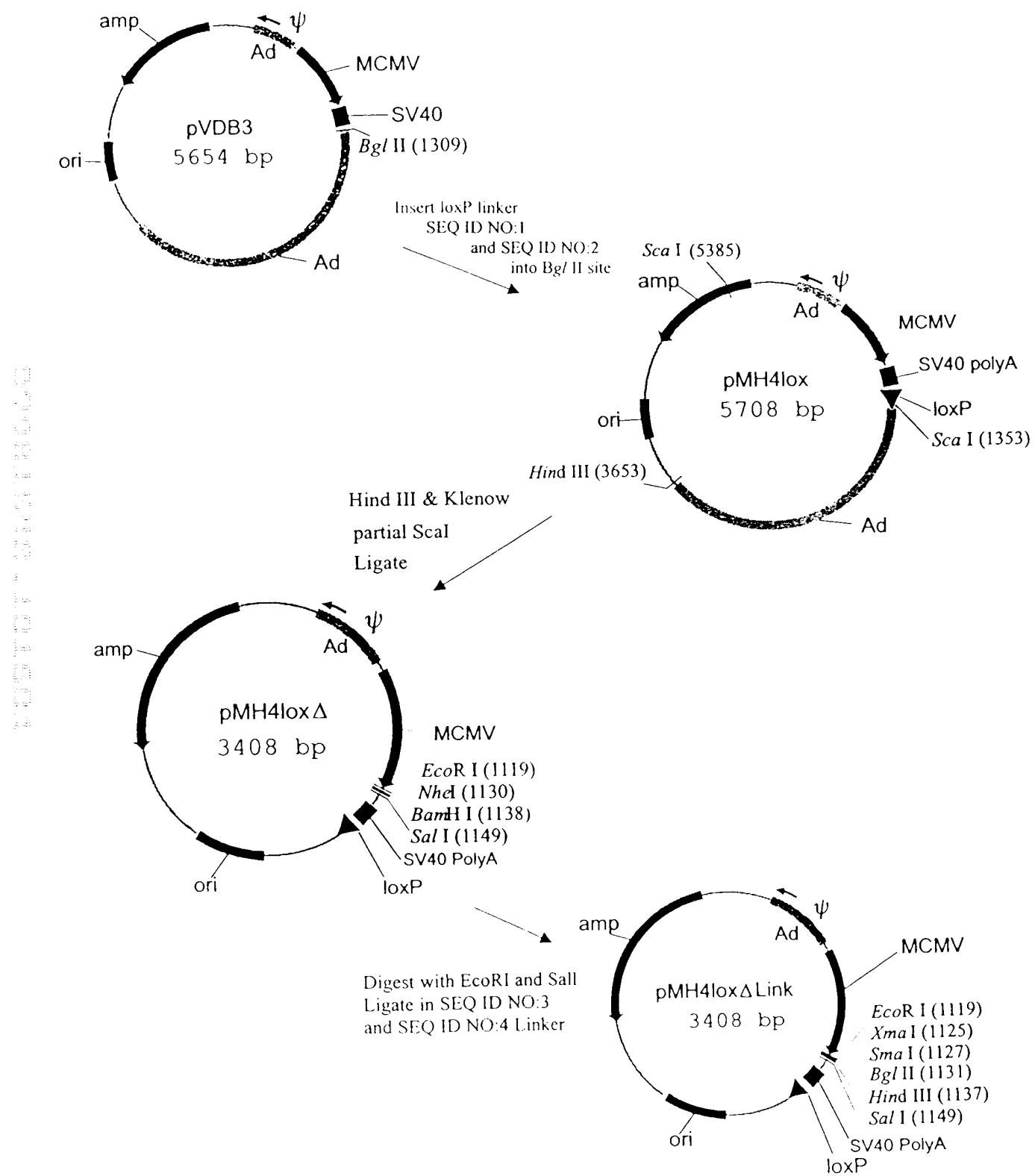


Fig. 6A

# CONSTRUCTION OF A SHUTTLE PLASMID CONTAINING A pUC DERIVED ORIGIN

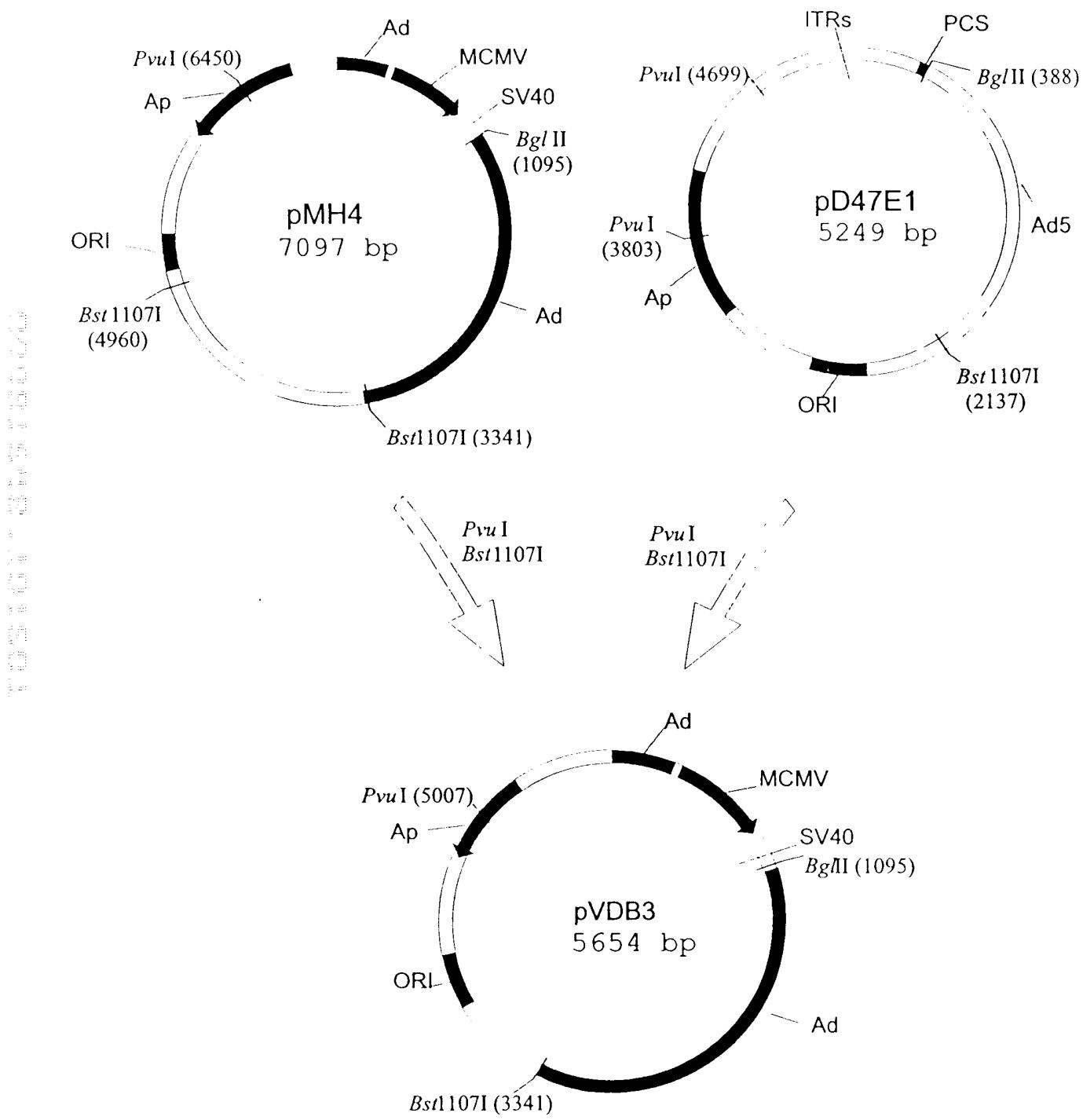


Fig. 6B

# CONSTRUCTION OF HCMV loxP PLASMIDS FOR RESCUE OF EXPRESSION CASSETTES

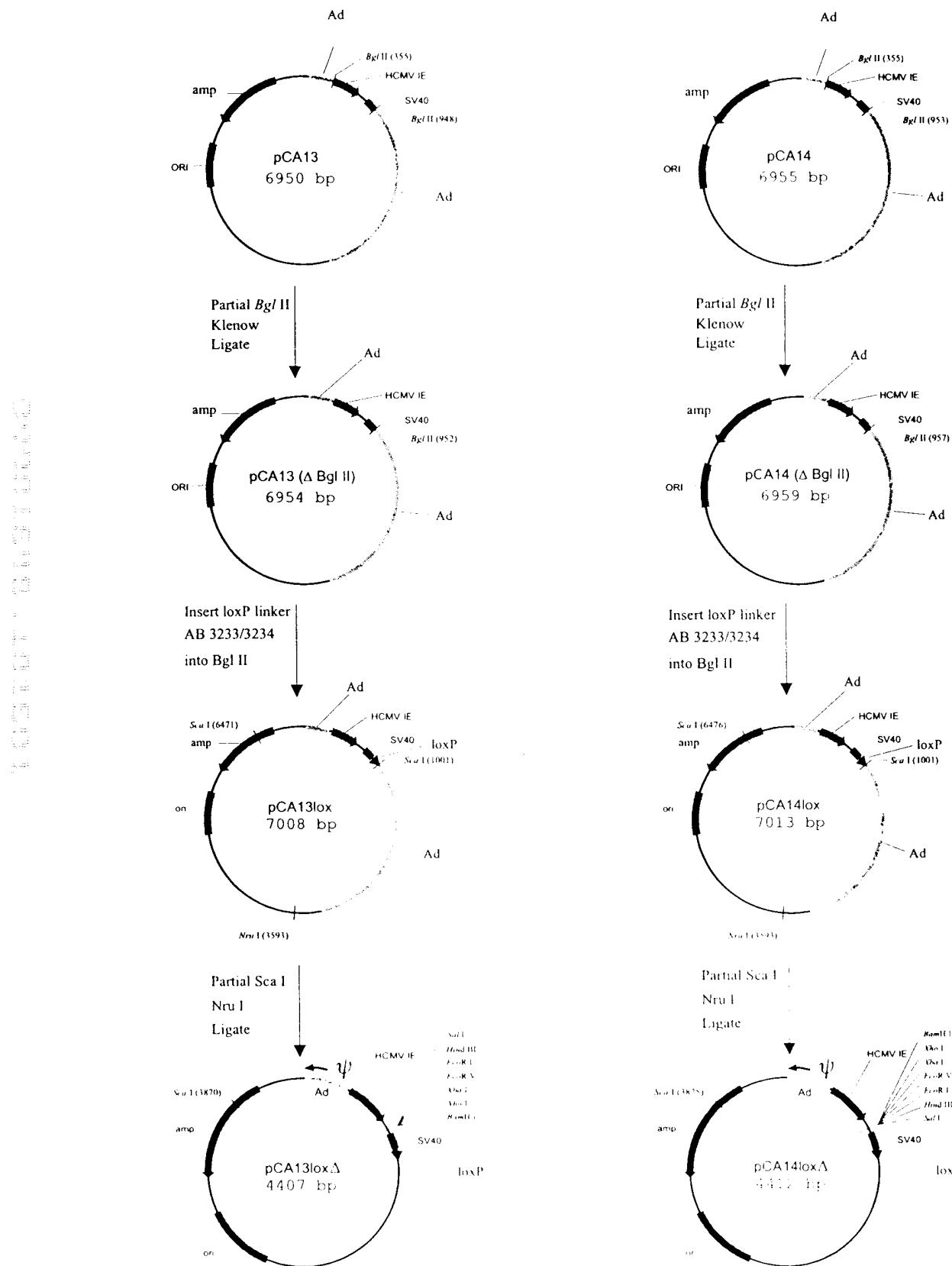


Fig. 7

# CONSTRUCTION OF pCA36LOX and pCA36LOX $\Delta$ SHUTTLE PLASMIDS FOR RESCUE OF LACZ

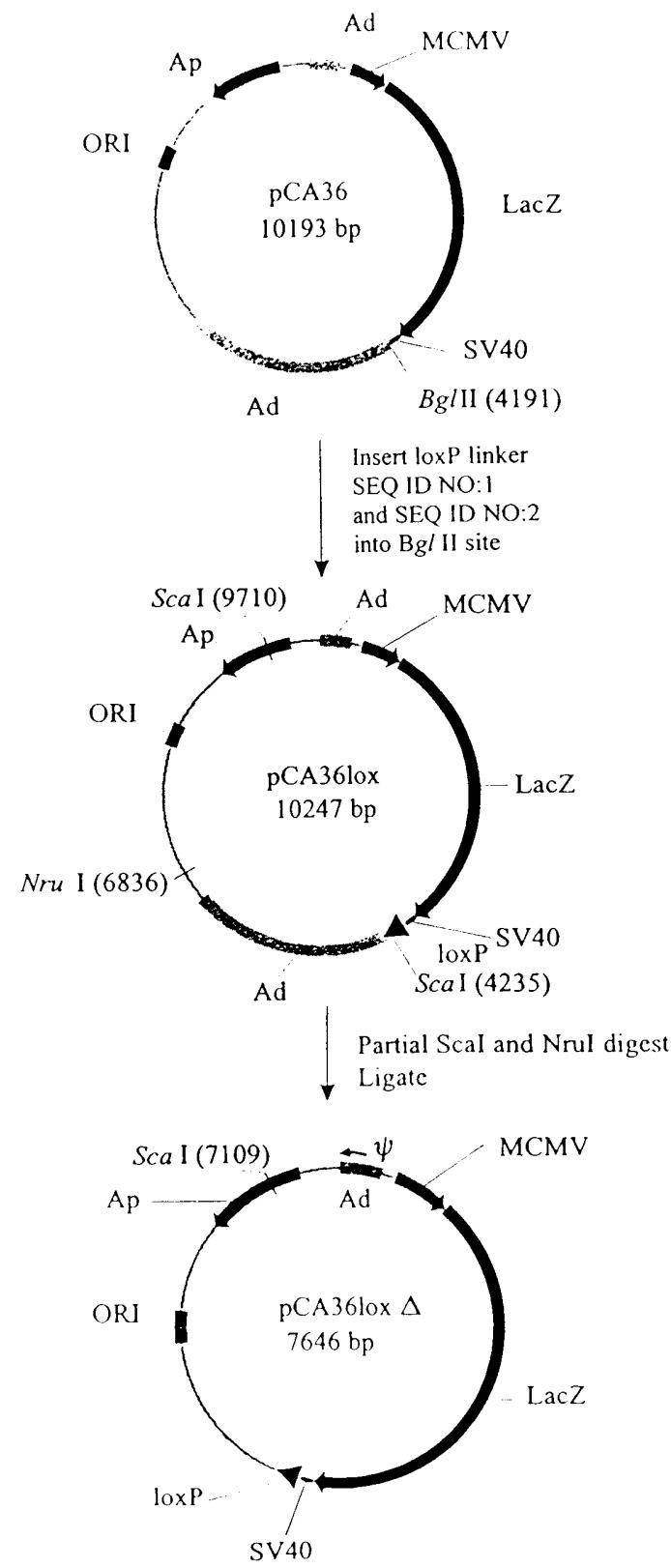


Fig. 8A

Cotransfection of 293Cre cells with AdLC8c DNA-TP and a shuttle plasmid containing a loxP site for generation of Ad expression vectors

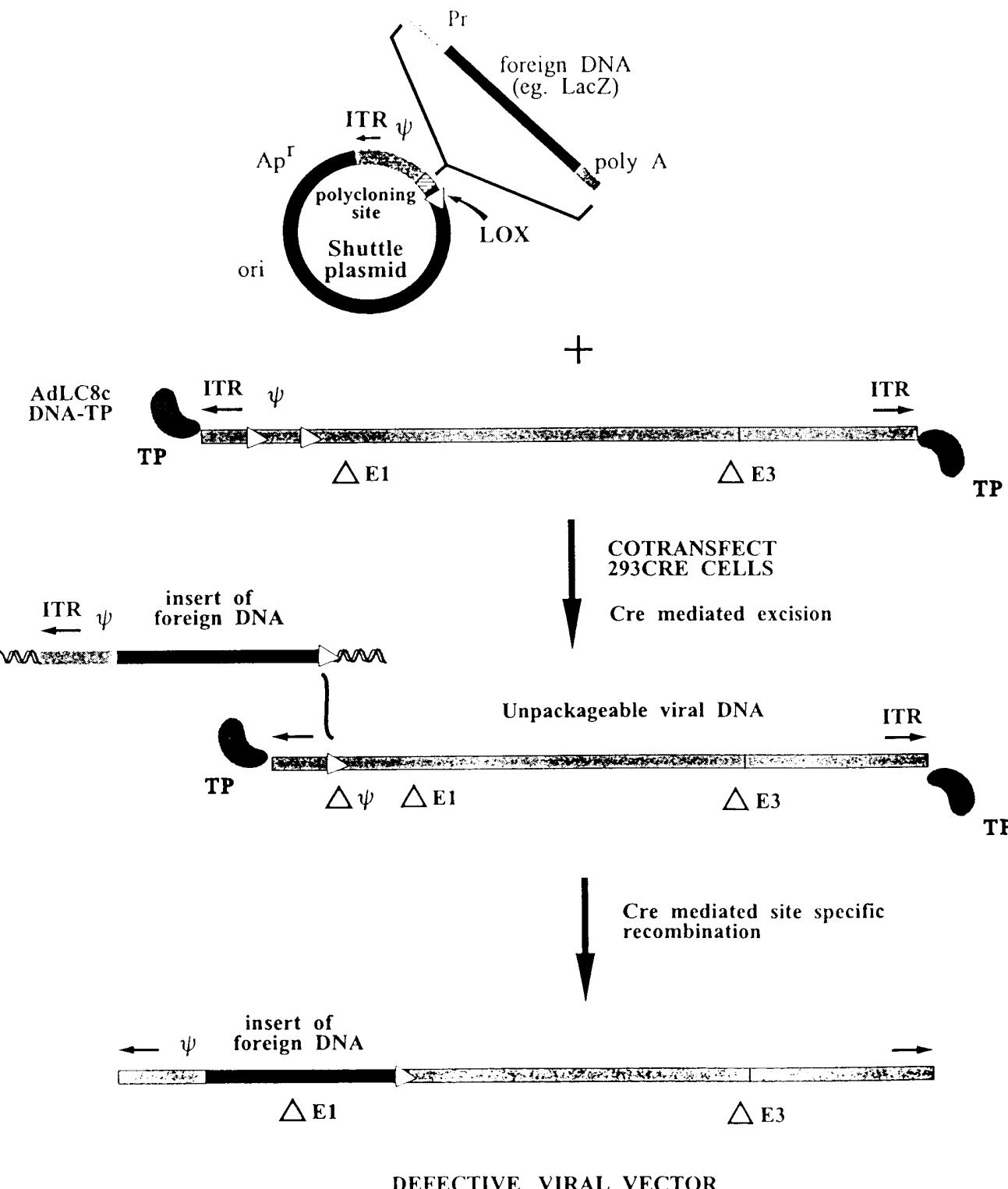


Fig. 8B

Cotransfection of 293Cre cells with restricted AdLC8c DNA-TP and loxP shuttle plasmid for generation of Ad expression vectors

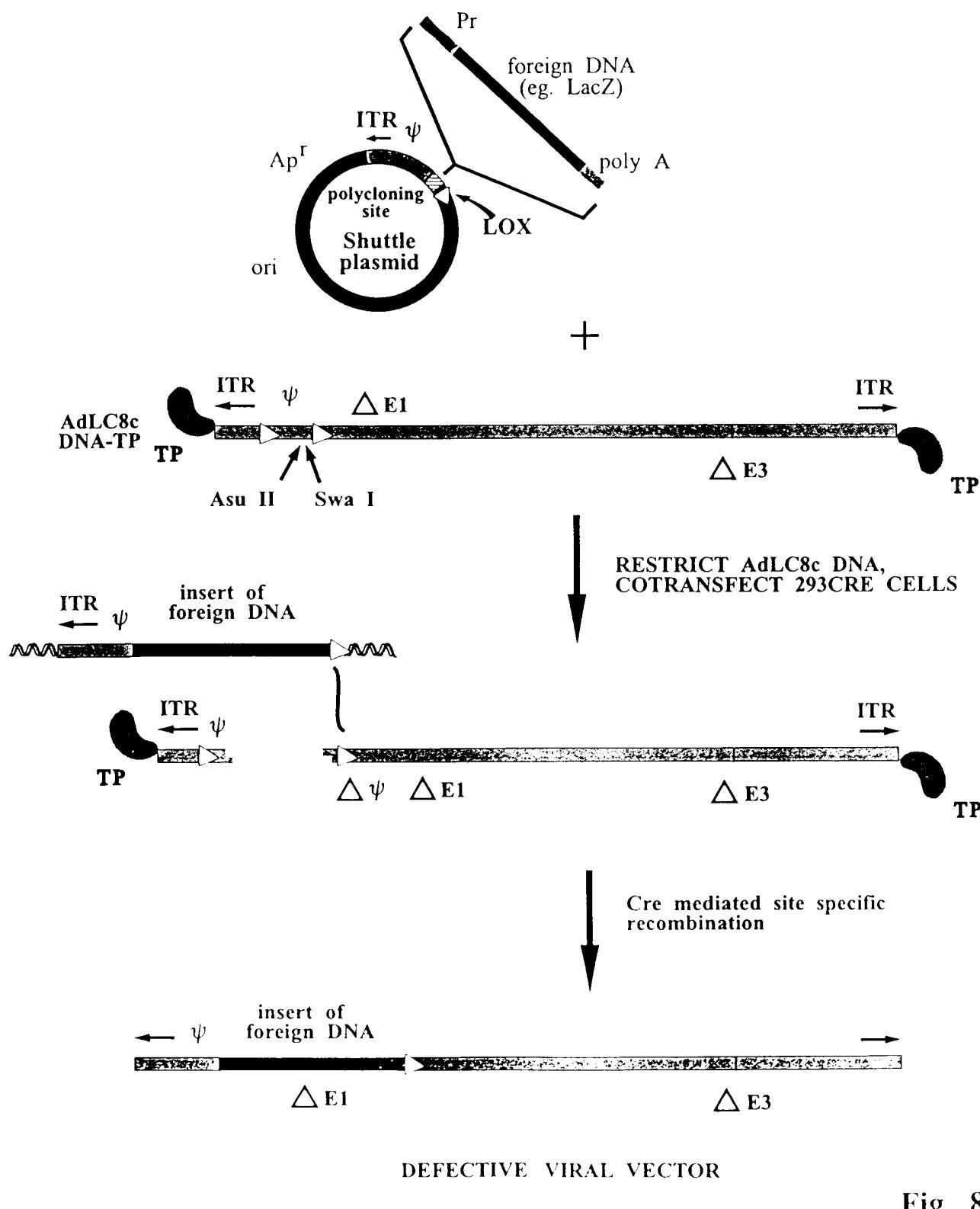


Fig. 8C

## CONSTRUCTION OF SHUTTLE PLASMIDS EXPRESSING Cre

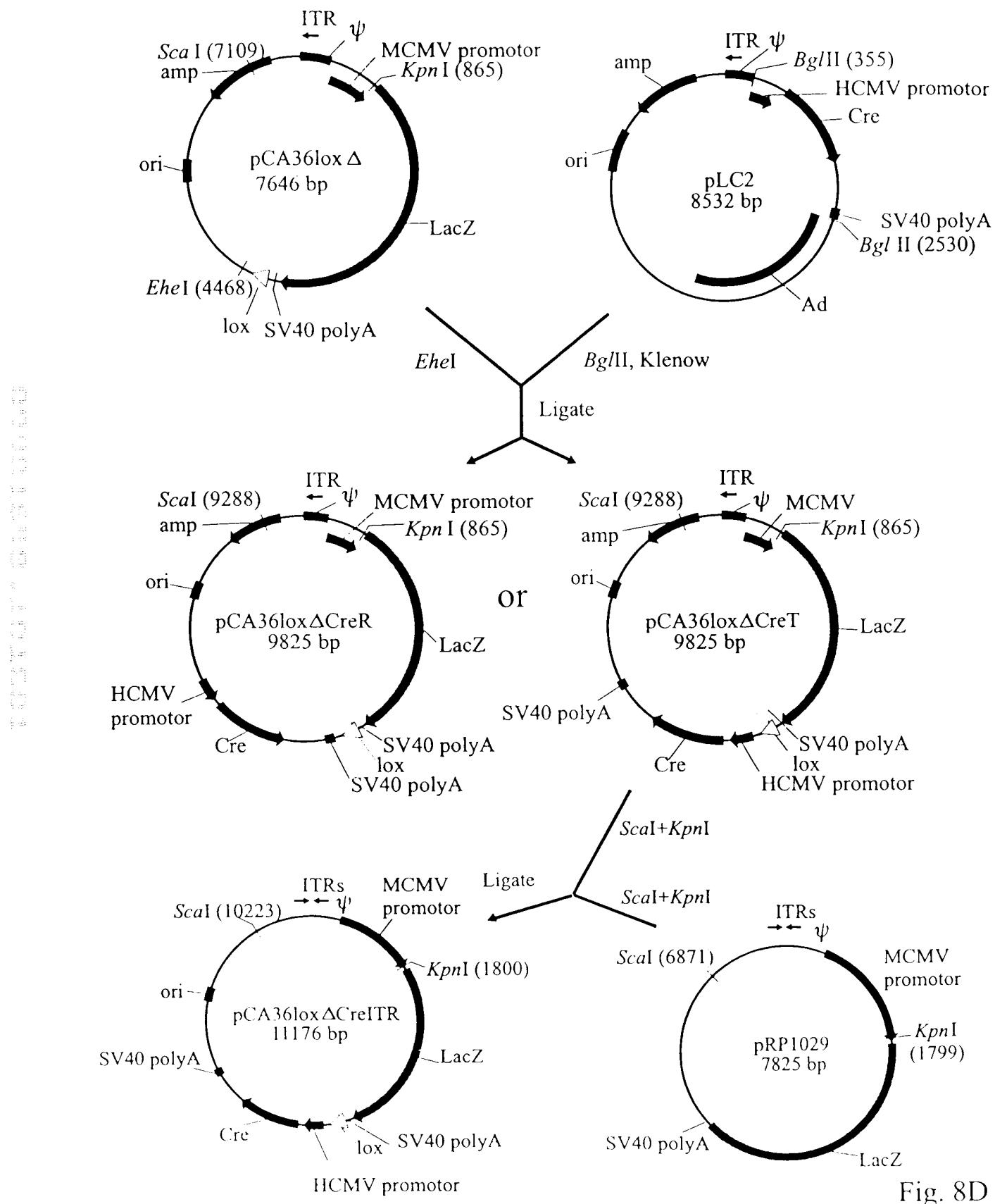


Fig. 8D

**Cotransfection of 293 cells with pBHG10lox and a "Lox" shuttle plasmid expressing Cre for generation of Ad expression vectors**

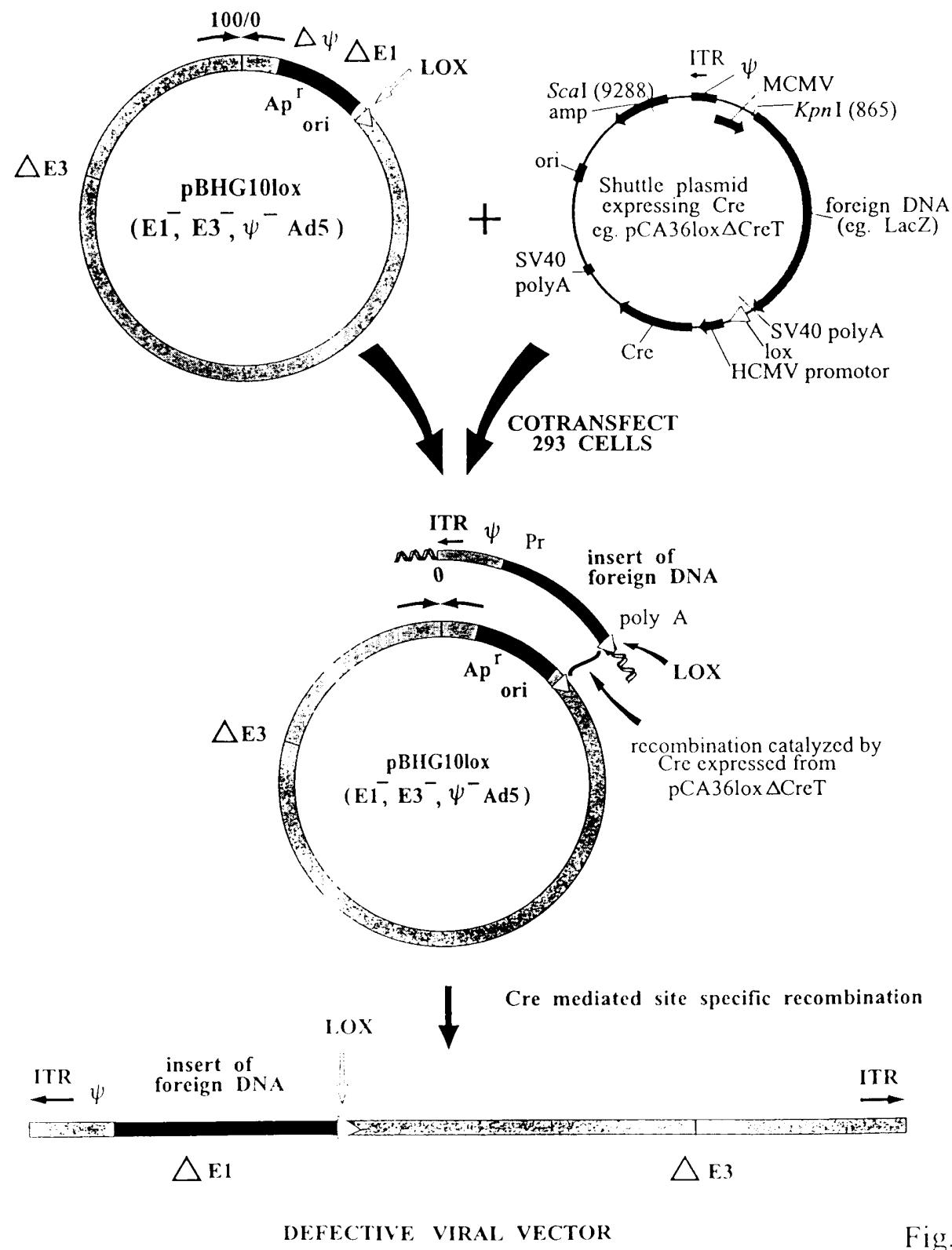


Fig. 8E

## CONSTRUCTION OF Ad GENOMIC PLASMID ENCODING CRE

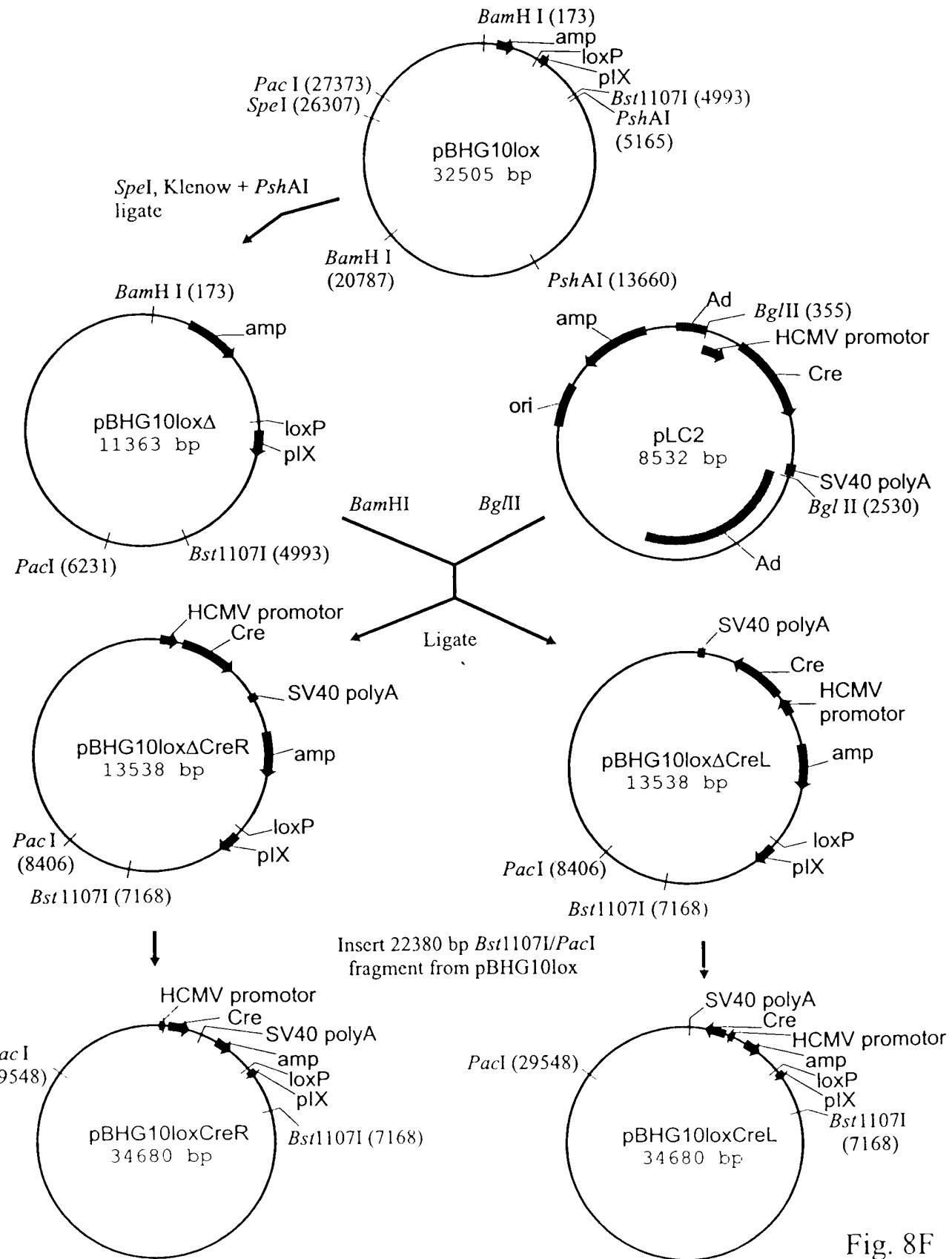


Fig. 8F

# RESCUE OF FIBRE MUTATIONS USING CRE/LOX RECOMBINATION

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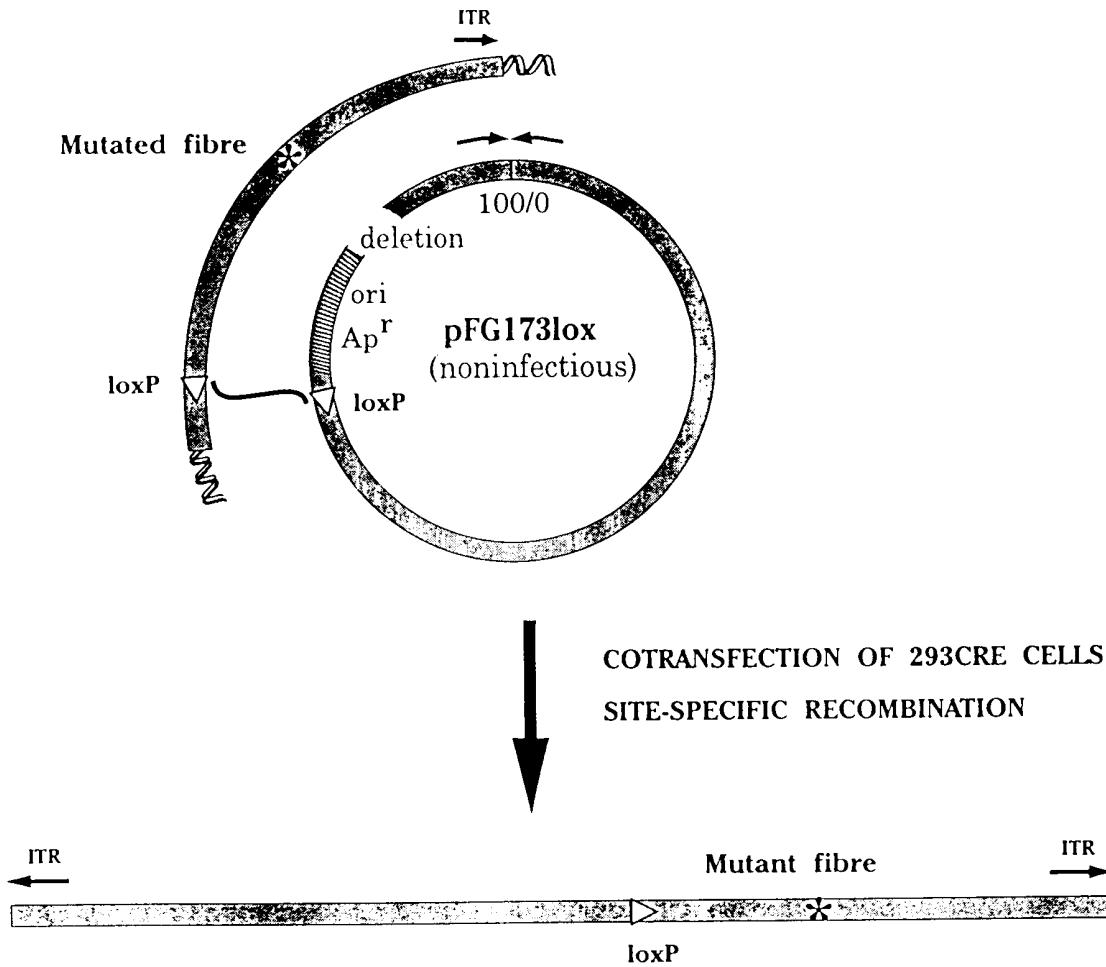


Fig. 9A

## CONSTRUCTION OF pAB14lox $\Delta$

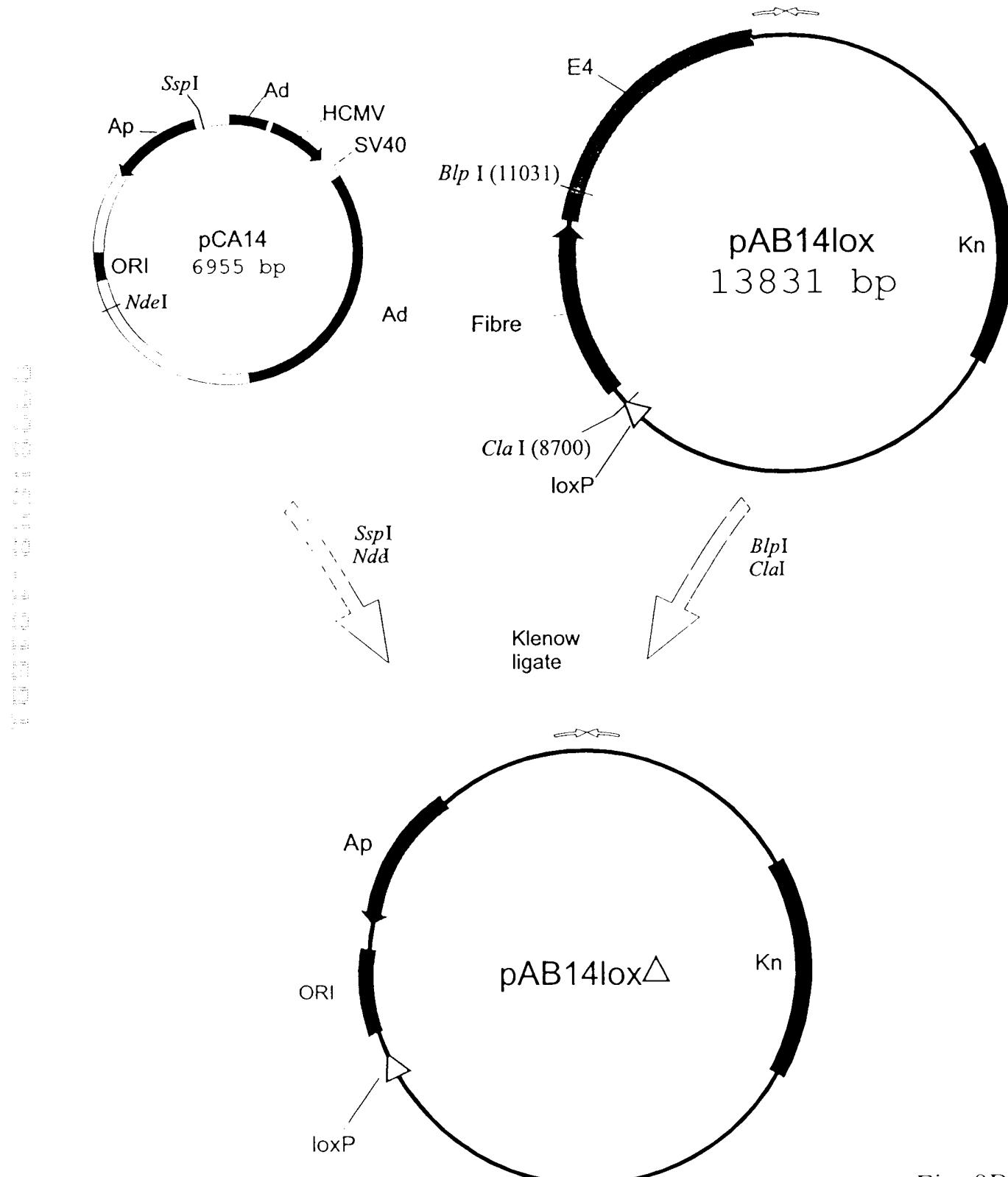
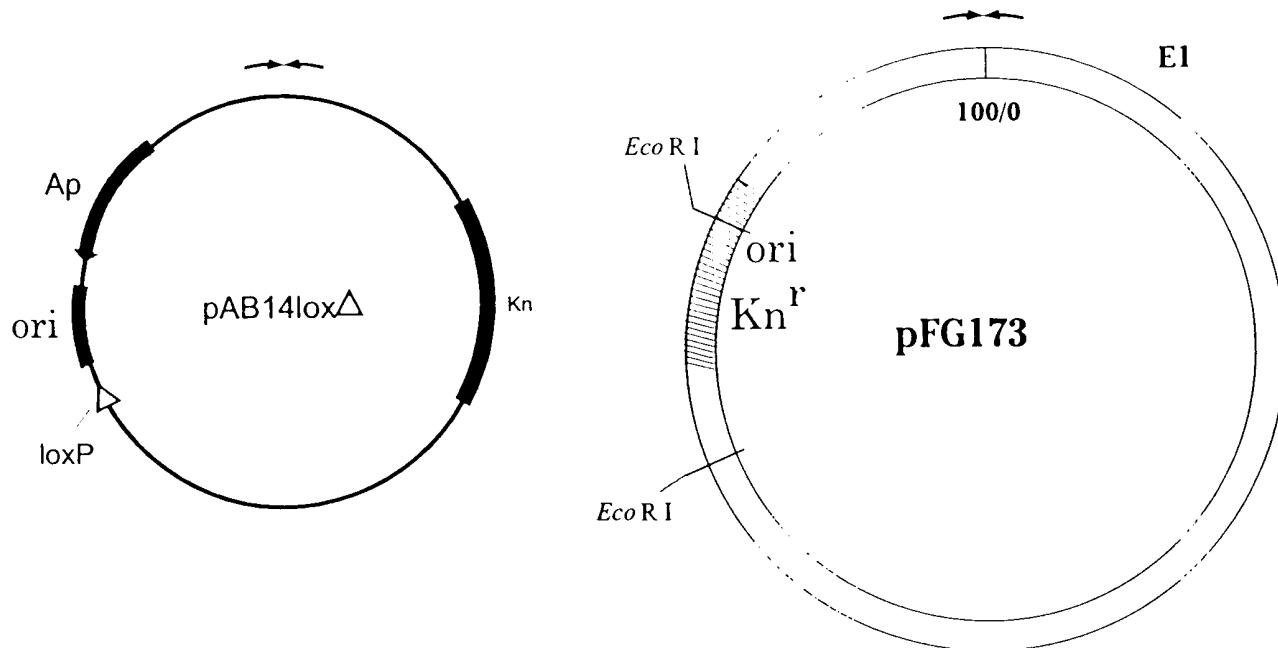


Fig. 9B

# CONSTRUCTION OF pFG173lox



Restriction, transformation of *E. coli*,  
homologous recombination

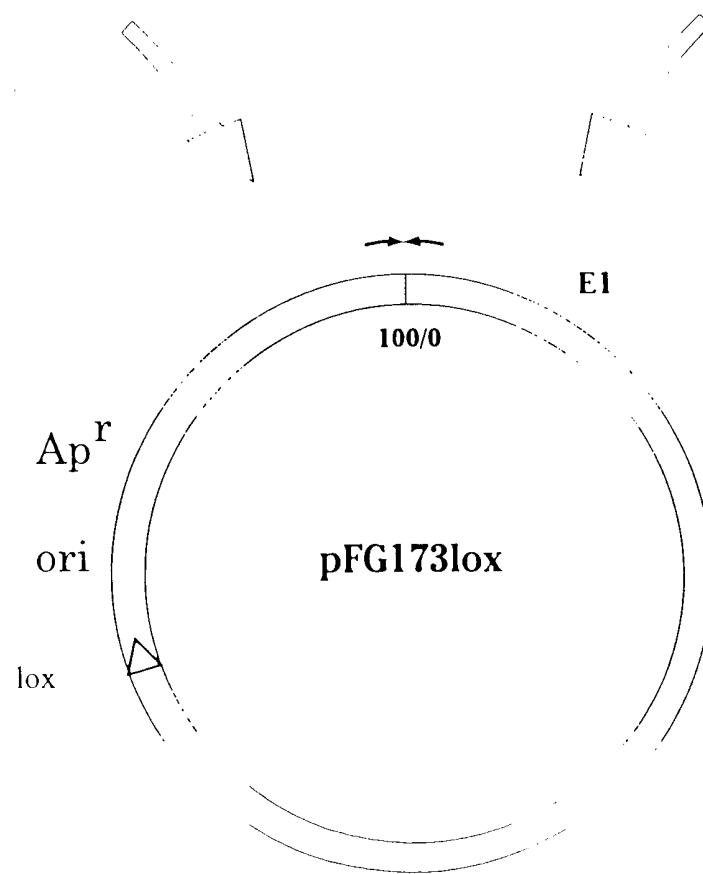


Fig. 9C

# CONSTRUCTION OF pFG23dX1lox AND pFG23dX1lox<sub>c</sub> FOR RESCUE OF MUTANT FIBRE INTO AD VIRUS

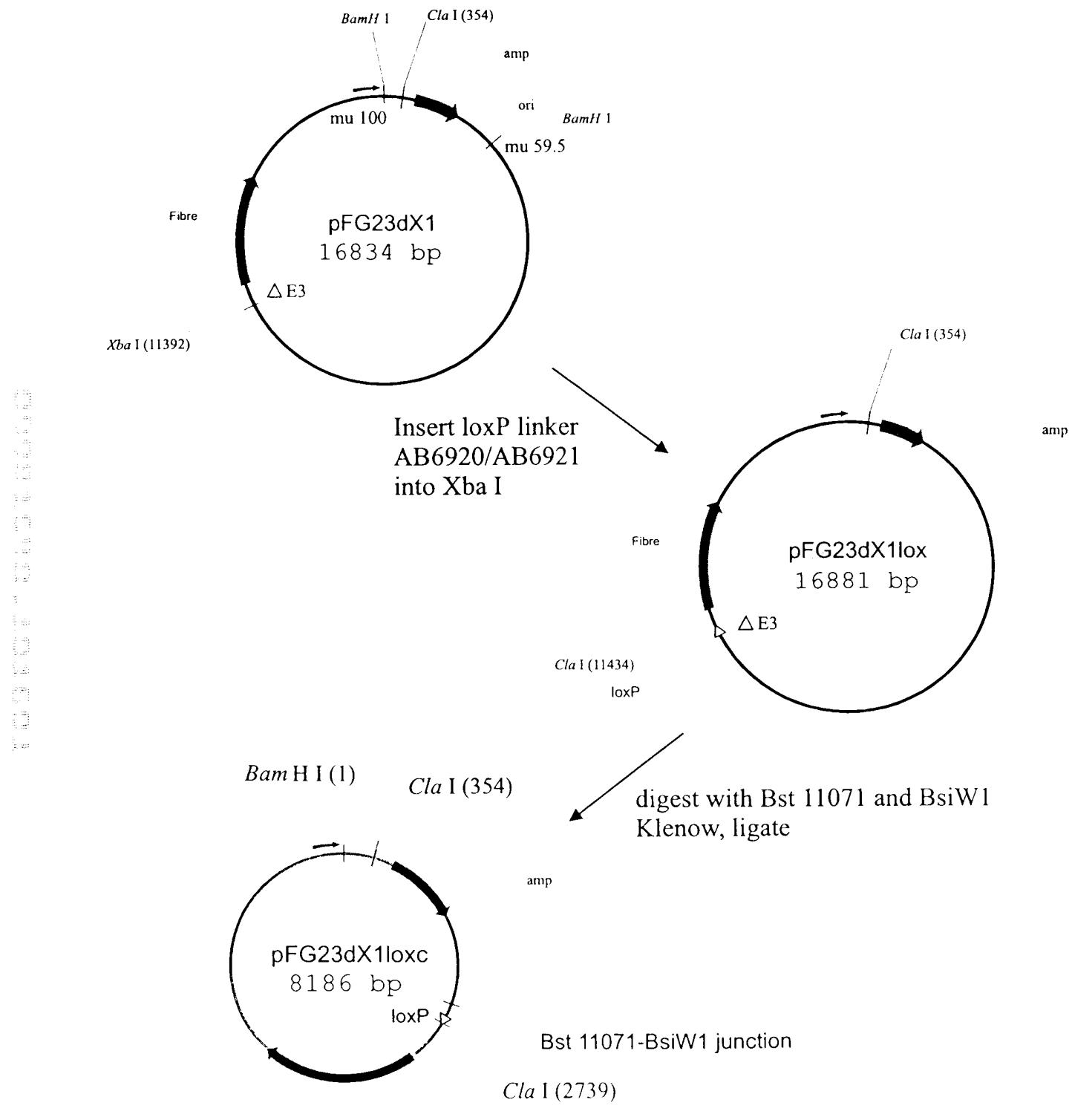


Fig. 10

# A PLASMID FOR RESCUE OF A FOREIGN DNA INTO AD VIRUS

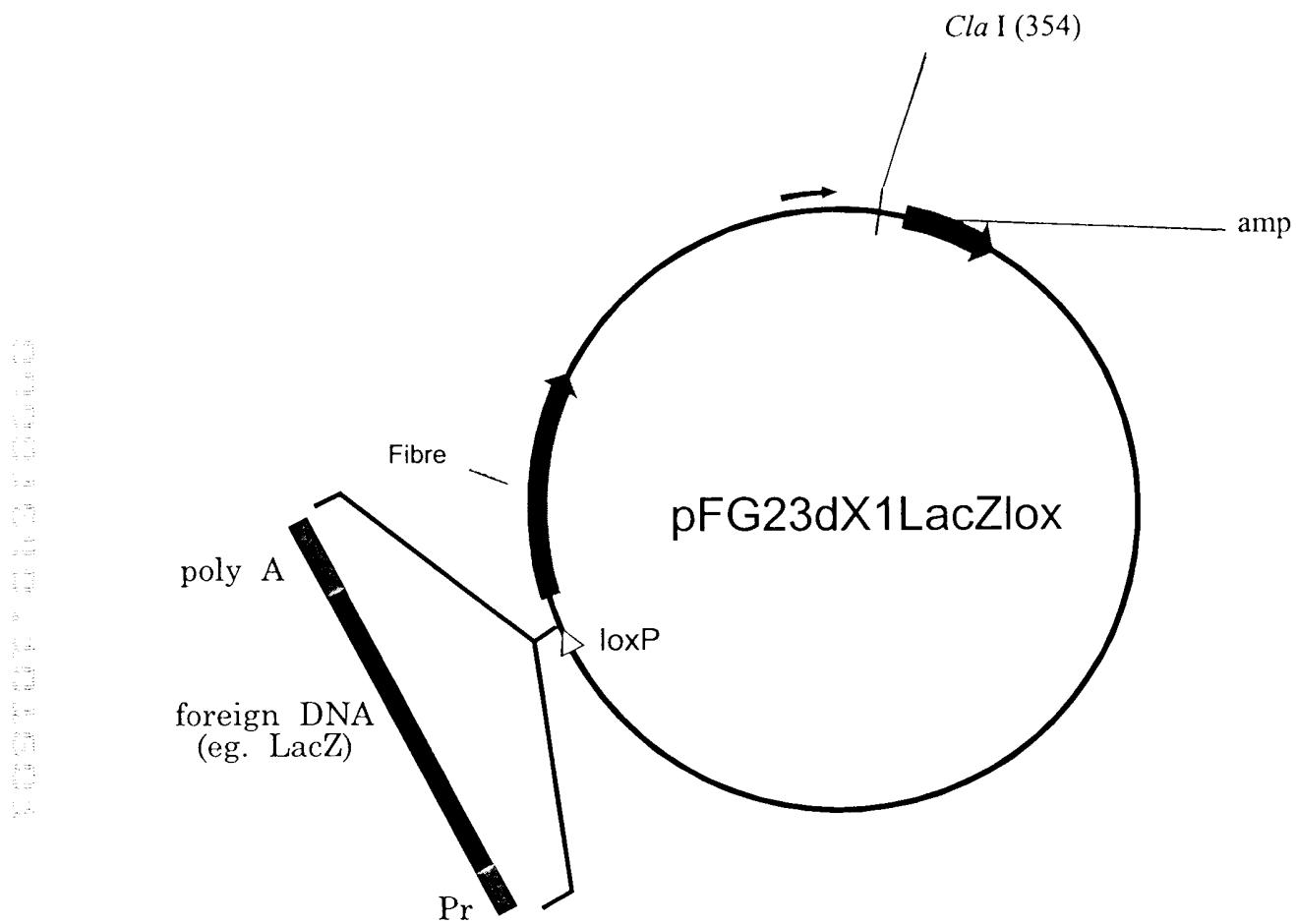


Fig. 11A

## RESCUE OF FIBRE MUTATIONS USING CRE/LOX RECOMBINATION

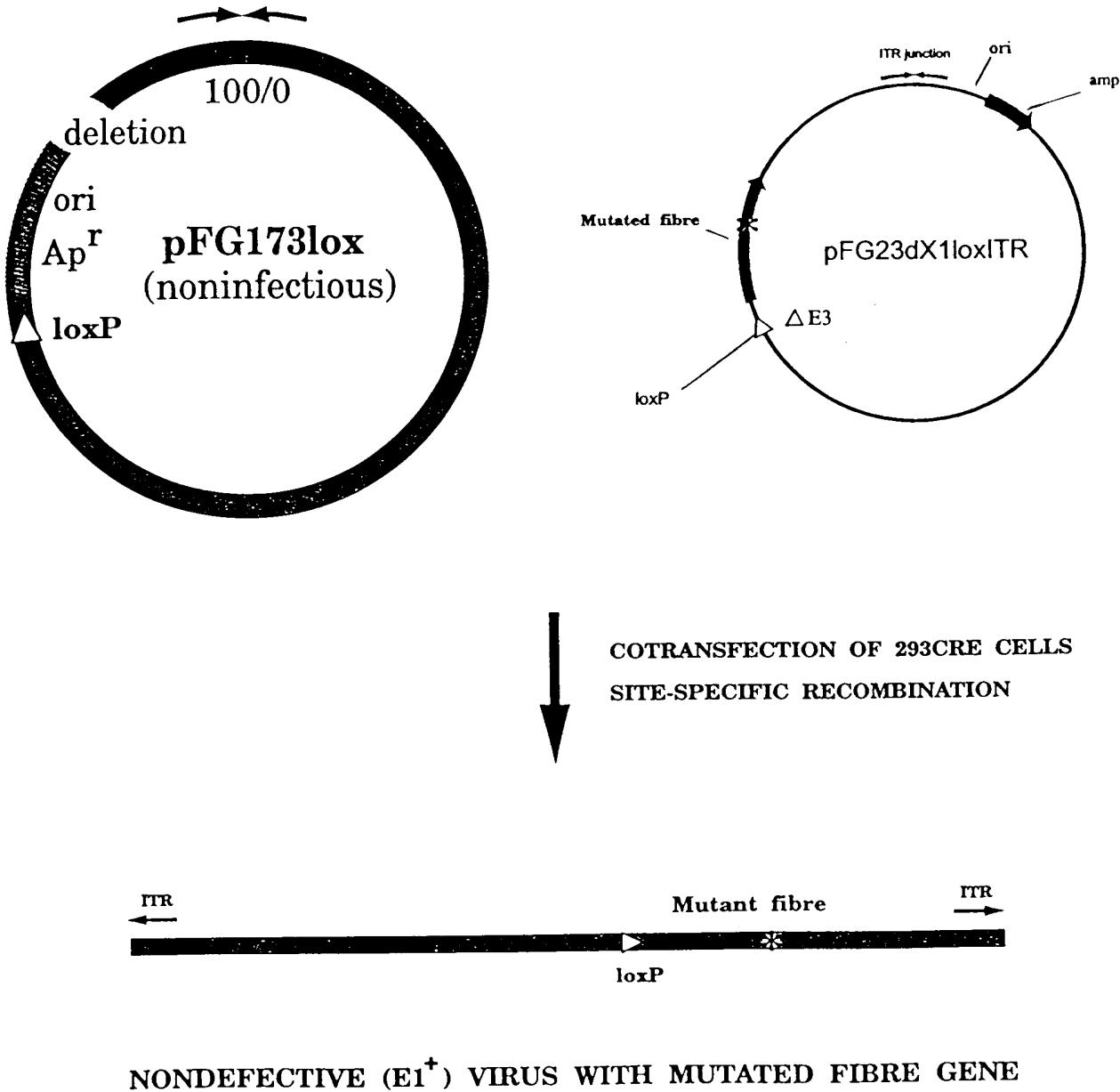
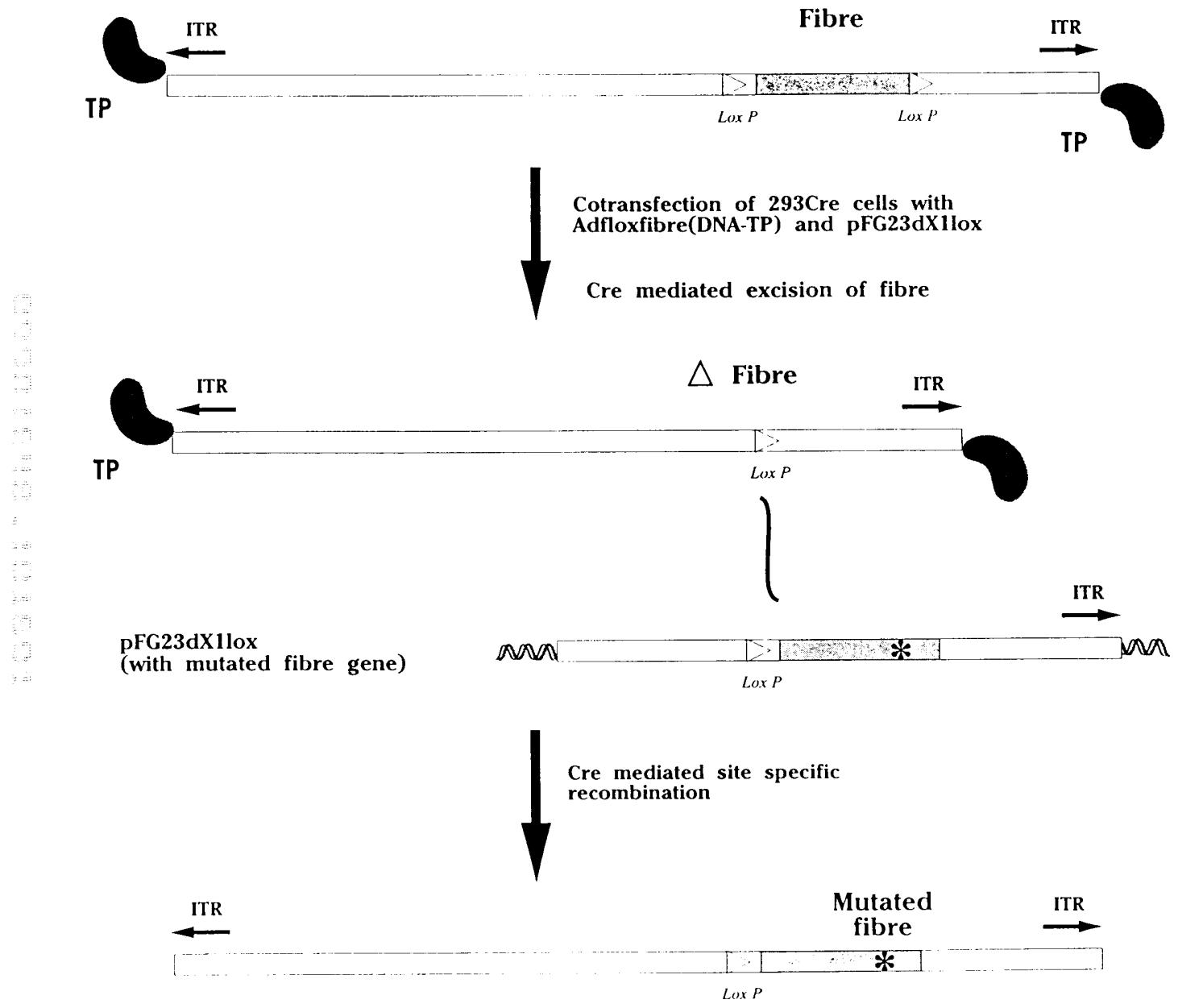


FIGURE 11B

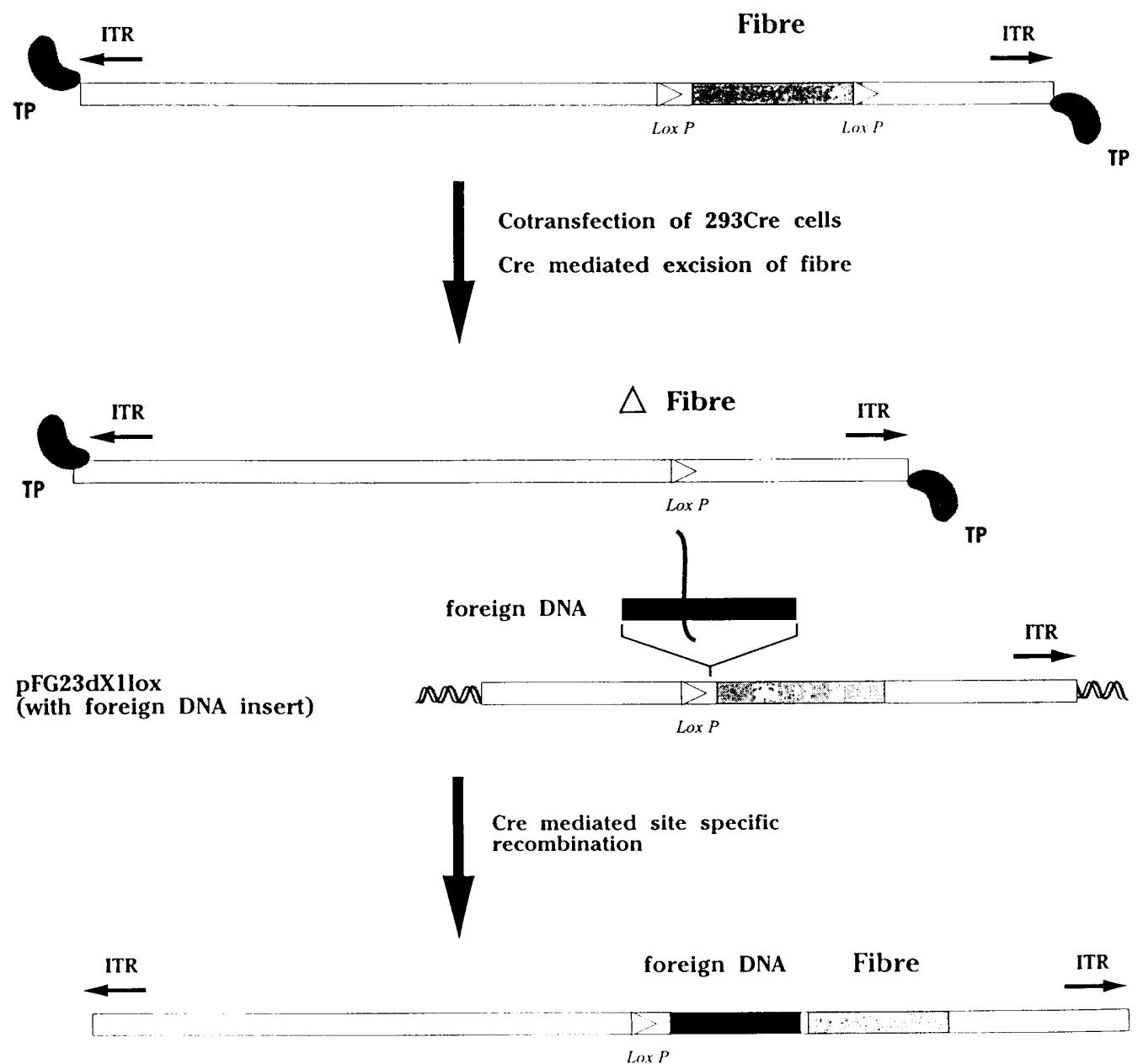
**Isolation of a virus containing a mutant fibre gene  
by Cre-lox recombination using DNA-TP and cotransfection**



RECOMBINANT VIRUS CONTAINING A MUTATED FIBRE GENE

Fig. 12

# Isolation of a virus containing a foreign DNA insert upstream of the fibre gene by Cre-lox recombination



RECOMBINANT VIRUS CONTAINING AN INSERT OF FOREIGN DNA  
UPSTREAM OF THE FIBRE GENE

Fig. 13

# CONSTRUCTION OF pAB14FL0X FOR ISOLATION OF AN AD VIRUS WITH A FLOXED FIBRE GENE

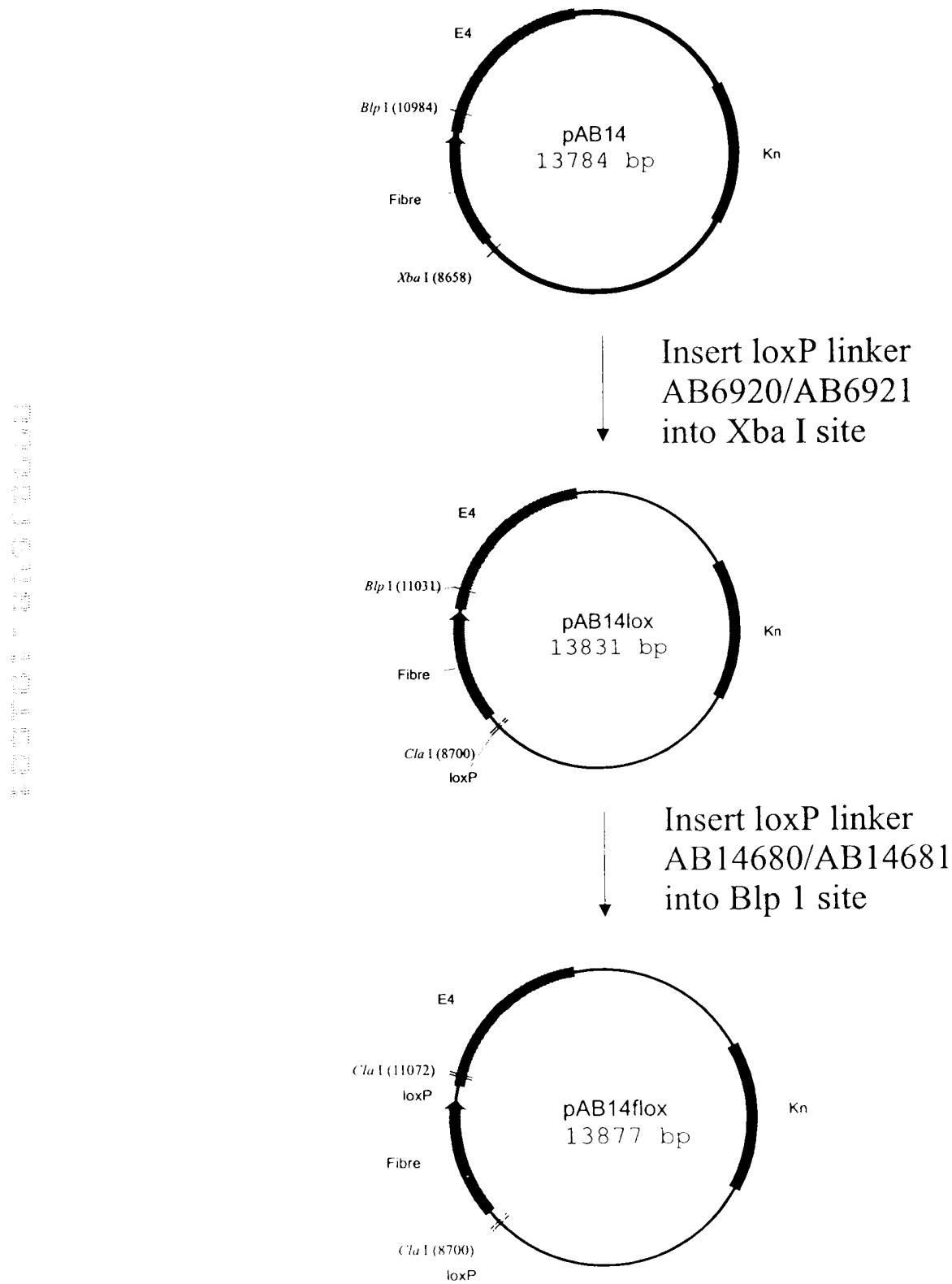


Fig. 14

# Isolation of a virus containing a fibre gene with flanking lox P sites.

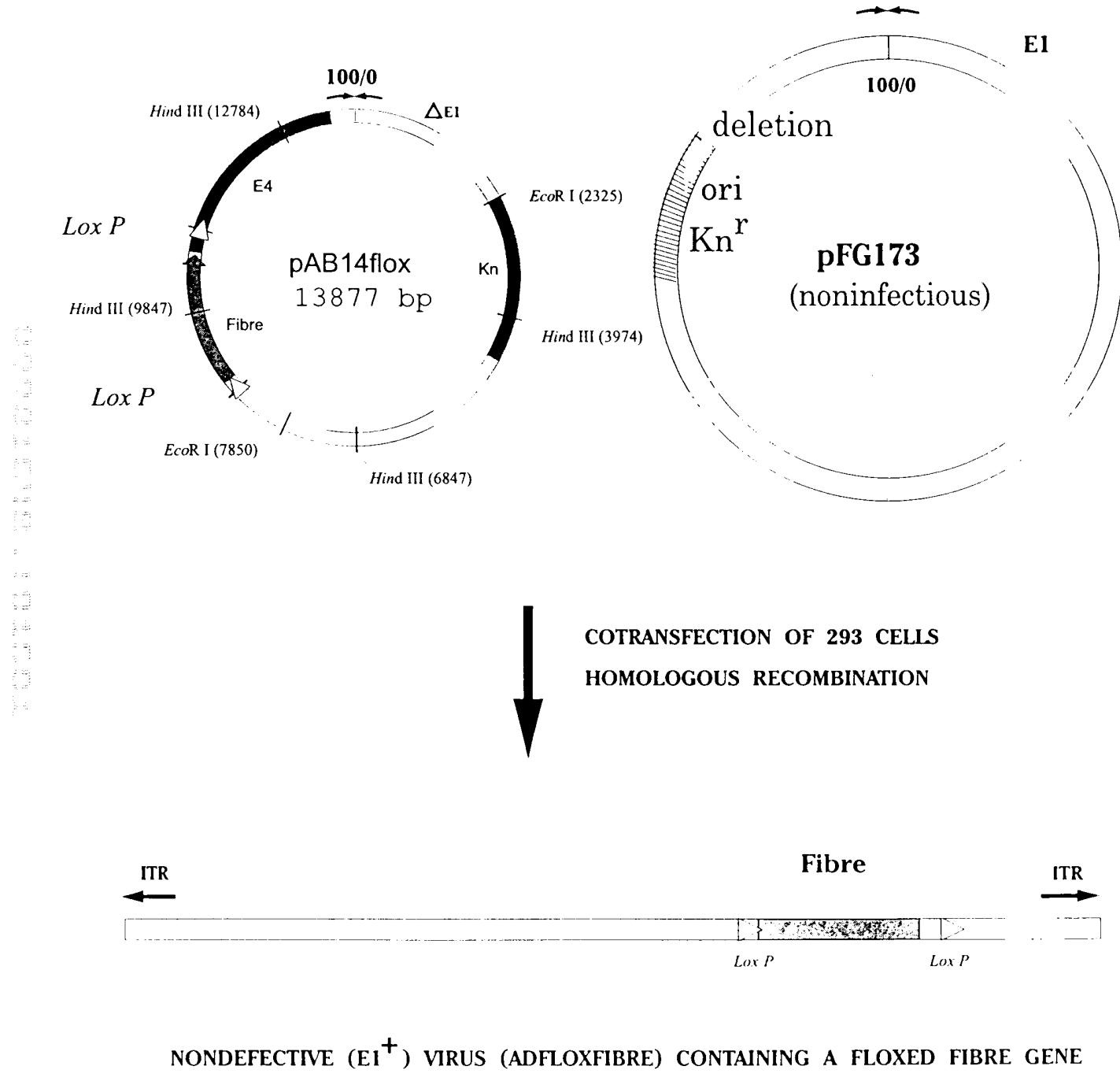


Fig.15